Hospital for Children and Adolescent
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PREMATURITY AND ATOPY

by

Mirjami Siltanen

ACADEMIC DISSERTATION

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SUMMARY

Recent evidence suggests that early life events have a central, life-long role in atopic sensitization. The effect is based on the postnatal conversion in T helper (Th) cell balance. During pregnancy the maternal and foetoplacental Th balance is Th2-deviated, after which it polarizes to the normal Th1 cell-dominant profile. Priming of the immunological memory is assumed to occur during this period of conversion.

Prematurely born infants often have at birth a severely immature immune and intestinal system. Antigen exposure during their neonatal period also diverges markedly from that of infants born at term. Thus, based on the current theory of the importance of early life events in priming of immunological memory, the atopic predisposition of prematurely born children can be assumed to differ from that of an unselected population of children.

The aim of this study was to evaluate the association between prematurity and atopy from two perspectives. In the first part of the study, the atopic status of prematurely born children was examined and compared with that of children born at term. We thus invited 100 consecutive prematurely born children with very low birth weight (VLBW) (birth weight ≤1500 g) to participate, selecting as controls 96 age-matched schoolchildren (birth weight >2500 g). Of the preterm and full-term groups, 72% and 68%, respectively, opted to participate. Both groups were examined at a mean age of 10.1 years at an outpatient clinic. Data on atopy were collected with a questionnaire, by performing skin prick testing (SPT) and by measuring serum total and three allergen-specific IgE antibodies. We further measured serum eosinophil cationic protein (ECP); serum IgG and IgA isotype antibodies to whole cow's milk, β-lactoglobulin, α-casein and ovalbumin; and IgG antibodies to gliadin. IgG antibody levels to tetanus and diphtheria toxoids were measured to estimate the general antibody production capacity. Data on prenatal and neonatal events affecting children in the preterm group were collected from hospital records.

Results showed that prematurity was linked to a decreased long-term risk of atopic sensitization. By age 10 years, children born preterm had significantly less atopy than their full-term peers; 15% versus 31% were defined as having had obvious atopy (atopic symptoms in at least one organ and at least one objective finding of an atopic

reaction) (odds ratio (OR) 0.41, 95% confidence interval (CI) 0.18-0.93, p=0.03). The mean value of serum total IgE was significantly lower in the preterm group, 41 kU/l versus 74 kU/l (p=0.02). In SPT, the children born full-term had positive reactions two to three times more often; 37% versus 17% had at least one positive reaction (p=0.007).

The difference in immune response between preterm and full-term children was also seen in food antigen responses. Children born preterm had markedly lower levels of antibodies to milk and its protein fractions ($p<0.001$ for IgA and IgG antibodies to cow's milk and α-casein and for IgG antibodies to β-lactoglobulin). IgG gliadin antibodies were also significantly lower in the preterm group (p=0.03), while for IgG ovalbumin antibodies the difference was not significant. In the preterm group, both those born before gestational week 30 and those given milk formula early (before day 50) had the lowest levels of milk antibodies. In the preterm group, atopy was associated with low levels of IgG milk antibodies but high levels of IgG ovalbumin antibodies. Thus, since the capacity to develop specific immunity against foreign antigens was shown to be compromised in preterm infants, it is likely that oral tolerance in this group is induced by early initiation of feeding, reflecting the effect of the immature gastrointestinal and immune systems. The presence of less atopy in these children is another marker of tolerance development.

Children born preterm had significantly more wheezing. The cumulative incidence of wheezing was 43%, as compared with 17% in children born full-term (OR 3.71, 95% CI 1.67-8.25, p=0.001). Wheezing was significantly associated with atopy in the full-term group but not in the preterm group (64% of full-term wheezers versus 23% of preterm wheezers were defined as atopic, p=0.024). In the preterm group, wheezing was associated with neonatal factors related to severe immaturity (low gestational age, respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD); p=0.039, $p<0.001$ and $p<0.001$, respectively). Current wheezing at the age of 10 years was, by contrast, no longer related to the above-mentioned neonatal variables but was instead associated with atopy; wheezers of the preterm group who had wheezed at age 10 were significantly more often atopic than those who no longer wheezed (62% vs. 9%, p=0.006). In spirometry testing, children born preterm exhaled significantly lower values of all measured variables. In the preterm group, wheezing, asthma and low gestational age, but not atopy, were significantly associated with lower lung function

values. The results show that the background of respiratory morbidity in preterm children differs markedly from that in an unselected population of children.

In the second part of the study, we analysed the possible association of maternal atopy with preterm birth. The study consisted of an inquiry about atopic symptoms and doctor-diagnosed atopic diseases of parents of 370 VLBW (birth weight <1500 g) children and of parents of 544 children born at term (birth weight >3000 g). The response rate was 56% for both groups. The trend test showed that the risk of maternal allergic rhinitis grew in parallel with infant's birth weight (p=0.03). The probability of mothers of extremely low birth weight (ELBW) infants (birth weight <1000 g) to have doctor-diagnosed allergic rhinitis was significantly lower (OR 0.49, 95% CI 0.26-0.89) than that of mothers of infants born at term. Fathers of infants of different birth weights showed no differences in prevalence of atopic symptoms. This finding suggests that the maternal atopy-related immune balance may have an effect on maintenance of pregnancy.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by their Roman numerals (I-IV):

I Siltanen M, Kajosaari M, Pohjavuori M, Savilahti E. Prematurity at birth reduces the long-term risk of atopy. J Allergy Clin Immunol 107:229-234, 2001.

II Siltanen M, Savilahti E, Pohjavuori M, Kajosaari M. Respiratory symptoms and lung function in relation to atopy in children born preterm. Pediatr Pulmonol 37:43-49, 2004.

III Siltanen M, Kajosaari M, Savilahti EM, Pohjavuori M, Savilahti E. IgG and IgA antibody levels to cow´s milk are low at age 10 years in children born preterm. J Allergy Clin Immunol 110:658-663, 2002.

IV Savilahti E, Siltanen M, Pekkanen J, Kajosaari M. Mothers of very low birth weight infants have less atopy than mothers of full-term infants. Submitted.

ABBREVIATIONS

ACAS	α-casein
APC	antigen-presenting cell
AU	arbitary unit
BLG	β-lactoglobulin
BPD	bronchopulmonary dysplasia
CBMC	cord blood mononuclear cell
CI	confidence interval
CLD	chronic lung disease
ECP	eosinophil cationic protein
ELBW	extremely low birth weight
ELISA	enzyme-linked immunosorbent assay
FEF25-75	forced midexpiratory flow of FVC
FEF50	forced expiratory flow after 50% of vital capacity has been exhaled
FEV1	forced expiratory volume in one second
FVC	forced vital capacity
HLA	human leucocyte antigen
IFN	interferon
IL	interleukin
OR	odds ratio
PBMC	peripheral blood mononuclear cell
RDS	respiratory distress syndrome
SPT	skin prick test
TCR	T cell receptor
Th	T helper cell
TGF	transforming growth factor
TNF	tumour necrosis factor
VLBW	very low birth weight

INTRODUCTION

An infant is defined as premature if gestational age at birth is less than 37 weeks. The proportion of preterm infants of all newborns in Finland during the last decade has been 5.4-6.3%. Very low birth weight (VLBW) infants have a birth weight of less than 1500 g, and extremely low birth weight (ELBW) infants of less than 1000 g. VLBW infants constitute 15% of all infants born preterm. In 2001, altogether 55 997 babies were born in Finland, 3373 of whom were preterm, with 494 having VLBW (National Birth Register).

During recent years the outcome of children born preterm has improved markedly. Of the cohort of ELBW infants born in 1996-1997 in Finland, 42% at 18 months' age were classified as normally developed and 18% as severely impaired (Tommiska et al. 2003). Thus, although a major part of even the most immature infants survive without impairment, severely preterm infants are still at risk for several specific impairments; including developmental delays, poor growth and neurosensory and cognitive impairments. Less is known of their status in relation to the most common diseases of childhood, the atopic diseases.

IgE-mediated allergic diseases, i.e. atopic diseases (atopic eczema, allergic rhinitis, allergic asthma), form a common health problem among otherwise healthy children in many western countries. By age 14, an average of 40% of Finnish children have had some atopic symptoms (Pekkanen et al. 1997). The causes underlying the increasing prevalence of atopic diseases remain obscure. However, early childhood has long been thought to form a critical period for the development of immunological memory, because after birth the T helper (Th) cell balance converts from pregnancy-related Th2 cell-type dominance (Wegmann et al. 1993) to Th1 cell-type profile under the influence of genetic and environmental factors. This occurs in "normal" non-atopic infants during the first year (Prescott et al. 1999). This period of conversion is now assumed to form the window of opportunity for atopic sensitization.

Early life events differ significantly between infants born preterm and those born at term. Severely preterm children stay in a hospital for their entire neonatal period and need intensive care treatment, including respiratory support, intravenous nutrition and

repeated antibiotic treatments for infections. Preterm infants are also exposed to dietary antigens earlier than full-term infants. Thus, preterm infants encounter an exceptional environment with an immature immune system, which may have long-term effects on their immune system. The specific environmental factors encountered by preterm infants in early life are included in those assumed to be important in development of atopic diseases (Strachan 1989, Holt et al. 1999).

Here, we evaluated whether immaturity at birth and subsequent environmental stimuli during early life are reflected in later atopic predisposition of the child. During the study we found evidence of interaction between maternal atopy and preterm delivery, which we further evaluated. Children born preterm do not form a large subgroup among atopic individuals. By examining their predisposition to atopy, we can, however, enhance the common understanding of early immunological mechanisms behind sensitization.

REVIEW OF THE LITERATURE

DEFINITIONS OF ATOPIC DISEASES AND NEONATAL CHRONIC LUNG DISEASE

Atopic diseases consist of inflammatory diseases with organ-specific symptoms such as atopic dermatitis, allergic rhinitis or rhinoconjunctivitis and allergic asthma. Atopic eczema or atopic dermatitis is defined as a chronic, relapsing, itching, inflammatory skin disease with typical morphology and distribution (Hanifin and Rajka 1980, Leung 2000). Atopic eczema manifests in 90% of subjects during the first 5 years of life and frequently precedes the development of allergic rhinitis or asthma (Leung 2000).

Allergic rhinitis is a combination of one or more of the following nasal symptoms: sneezing, itching rhinorrhoea and nasal congestion (Skoner 2001). When rhinitis is accompanied by itchy and watery eyes, the term allergic rhinoconjunctivitis is used. Allergic rhinitis frequently precedes the development of asthma; similar pro-inflammatory mediators, T helper (Th) 2 cell cytokines, chemokines and adhesion molecules are found in the nasal and bronchial epithelium of subjects with allergic rhinitis and those with asthma (Bousquet et al. 2001).

Martinez and Helms (1998) describe asthma as an inflammatory airway disease, a heterogeneous group of wheezing conditions manifesting as recurrent, reversible symptoms of bronchial obstruction. The definition of the Global Initiative for Asthma (National Institutes of Health 1995) states that ”Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils and T lymphocytes. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night and/or in the early morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli”. In the Finnish asthma programme (Haahtela and Laitinen 1996), asthma is defined as “an inflammatory disease of the

bronchi, marked by increased numbers of inflammatory cells such as mast cells and eosinophilic white blood cells. In asthmatic individuals, inflammation causes symptoms including obstruction of the bronchi of varying degrees which subsides either spontaneously or in response to therapy. Inflammation increases the sensitivity of the bronchi to many irritants."

Of all young children experiencing recurrent wheezing, only a minority goes on to develop persisting atopy-related asthma. Martinez et al. (1995) specified three types of wheezing in children under 6 years of age: transient early wheezing with wheezing before 3 years of age only, persistent wheezing with symptoms both before 3 years and after 3 years, and late-onset wheezing with symptoms first apperaring after 3 years. A considerable proportion of bronchial obstruction in infants was found to be transient early wheezing, commonly manifesting with viral respiratory infections. Transient early wheezing was associated with diminished airway function at birth, before manifestation of any wheezing, and was not linked to asthma or allergies later in life. By contrast, persistent and late-onset wheezing were found to be associated with an increased risk of persistent asthma and allergies (Martinez et al. 1995). The same applies to asthma in older children and adolescents; asthma in these groups has been demonstrated to be closely associated with atopic diathesis, reflected in IgE responses (Burrows et al. 1989, Sears et al. 1991, Peat et al. 1996).

Neonatal chronic lung disease is defined here because in a notable portion of children born preterm it causes long-term pulmonary consequences, including asthma-like symptoms. Neonatal chronic lung disease, designated first as bronchopulmonary dysplasia (BPD), was described by Northway et al. (1967). Later, Bancalari et al. (1979) defined BPD as a chronic lung disease in infants who had been mechanically ventilated, who had clinical signs of chronic respiratory disease, who required supplemental oxygen for more than 28 days and who had typical changes in chest radiograph. Because this definition includes a wide range of infants with varying gestational maturation and different outcomes, Shennan et al. (1988) created a new concept of a chronic lung disease (CLD), whereby an infant is considered to have CLD if continuous oxygen supplementation at 36 weeks´ gestational age is needed. Palta et al. (1998) evaluated different criteria of neonatal chronic lung disease in relation to late respiratory complications and concluded that radiographic evidence was more predictive of long-term respiratory outcome than other commonly used criteria. The term CLD is currently

most often applied to infants who after mechanical ventilation have persistent radiological changes and need supplemental oxygen for more than 28 days, or to those who have a supplemental oxygen requirement at the age of 36 weeks' gestation, while the term BPD is reserved for the most severe forms of lung damage (Bancalari 2002).

EPIDEMIOLOGY OF ATOPIC DISEASES AND PREMATURE BIRTH

Prevalence of atopic diseases in children

The prevalence of atopic diseases has increased in affluent countries (von Mutius 1998). In a worldwide study of prevalence of allergic diseases in children carried out in the 1990s, the differences were found to be 20- to 60-fold between countries of the highest and lowest prevalence (ISAAC Steering Committee 1998).

In Finland, four centres participated in the ISAAC study, and altogether 11 607 children aged 13-14 years were included. The study reported prevalences of asthma symptoms and asthma as follows (figures varying according to the geographic region): wheezing ever 27-33%, wheezing in past year 13-20%, asthma ever 4-8% and doctor-diagnosed asthma ever 4-7% (Pekkanen et al. 1997). The prevalence of allergic rhinoconjunctivitis and atopic eczema were as follows: rhinitis ever 44-55%, rhinitis in past year 33-46%, rhinoconjunctivitis in past year 15-23%, itching dermatitis 24-28%, eczema ever 23-26% and itching dermatitis in past year 17-22% (Remes et al. 1998). The cumulative incidence of atopy (atopy defined as a child ever having had atopic eczema or allergic rhinoconjunctivitis) was recorded to be 38-46% (Pekkanen et al. 1997). The highest frequencies were generally recorded in the Helsinki region. Compared with international figures, the 12-month prevalence of asthma in Finland was lower than in many other affluent countries, whereas the prevalences of allergic rhinoconjunctivitis and atopic eczema were among the highest in the world (ISAAC Steering Committee 1998).

Previous Finnish studies have reported a lower prevalence of allergic diseases in children and adolescents than that in the ISAAC study. Varjonen et al. (1992) carried out a study in 1989-1990 in the south-western part of Finland that evaluated frequencies of atopic diseases in 1712 adolescents aged 15-16 years. They reported the prevalence

of diagnosed asthma to be 2.5%, and prevalence of history of allergic rhinitis and atopic eczema to be 14% and 9.7%, respectively. In a multicentre study carried out in 1980 (3649 children aged 3-18 years), the prevalence of doctor-diagnosed asthma, allergic rhinitis and atopic eczema were 4.3%, 6.3% and 1.7%, respectively (Pöysä et al. 1991). These lower figures, compared with the results of the Finnish portion of the ISAAC study, are partly explained by different inclusion criteria; the figures included only doctor-diagnosed diseases.

Frequencies of food allergy have been difficult to determine because parents commonly report a range of symptoms as allergic. In a Norwegian study evaluating parentally perceived adverse reactions to food, the cumulative incidence of all reactions was 35% by age 2, and the cumulative incidence of reported adverse reactions to milk was 11.6% (Eggesbo et al. 1999), whereas cow's milk allergy diagnosed by a challenge test has only been found in about 2% of infants (Host and Halken 1990, Saarinen et al. 1999).

Different atopic symptoms frequently overlap. Varjonen et al. (1992) found that 55% of eczema patients concomitantly had allergic rhinitis and 7.8% had asthma; and 38% of children with rhinitis and 31% of children with asthma also suffered from atopic eczema. Another common feature of atopic diseases is a characteristic history in the onset of symptoms, referred to as an "atopic/allergic march". Atopic morbidity typically begins with atopic eczema and food allergies in infancy, followed by inhalant allergen sensitization, asthma and allergic rhinitis in later childhood (Kulig et al. 1999a, Rhodes et al. 2002).

Factors contributing to atopic sensitization

Atopic diseases are multifactorial illnesses determined by an interaction between genetic and environmental factors (Dold et al. 1992). Immaturity at birth may modify the effects of these factors by laying out a different immunological basis for postnatal development. The role of immaturity in atopic sensitization is reviewed in more detail in the section entitled "Atopy in prematurely born children".

During recent years the role of foetal growth in atopic diseases has been widely evaluated, inspired by the programming hypothesis of Barker (1998) (Godfrey et al. 1994, Fergusson et al. 1997, Gregory et al. 1999, Leadbitter et al. 1999, Shaheen et al.

1999, Katz et al. 2003). The theory states that foetal events lead to permanent effects on the structure and function of different organs, including among others, the immune system. The role of foetal disproportioned growth in subsequent development of atopic diseases remains, however, unestablished.

Genetic factors

Atopic diseases have a clear genetic basis . Family history is the most important risk factor for atopy (Tariq et al. 1998), but even its predictive capacity has proven to be low (Bergmann et al. 1997). The first molecular genetic studies have examined linkage between atopy and human leucocyte antigen (HLA) loci. Later, by candidate gene and positional cloning techniques, several other genes and genetic regions have also been linked to atopic diseases. The loci most frequently identified are on chromosomes 5, 6, 12 and 13 (Cookson and Moffatt 2000). However, as is the case with many other multifactorial disorders, efforts have failed to yield a consistent picture of genetic mechanisms.

Environmental factors

Nutritional proteins are among the first foreign antigens causing immune responses. Thus, the association between early feeding, especially cow's milk-based formula feeding, and atopy has been widely studied, but results have been inconclusive. In a number of studies, early avoidance of cow´s milk-based formula has been reported to offer protection against sensitization (Saarinen and Kajosaari 1995, Tariq et al. 1998, Oddy et al. 1999, Saarinen et al. 1999), at least in children at genetic risk of atopy (Mallet et al. 1992, Oldaeus et al. 1997, Siltanen et al. 2003). A recent review examining this issue also came to the same conclusion (Odijk et al. 2003). However, some studies have shown contrary results (de Jong et al. 2002, Sears et al. 2002).

Microbes, commensals as well as pathogens, have been suggested to have a major role in maturation of Th cell balance in early life. The theory of an inverse association between infection morbidity and atopic diseases was presented by Strachan (hygiene hypothesis) (1989). He analysed a large birth cohort of 17 414 subjects at 11 and 23 years of age and found a significant inverse association between sibship size and hay fever. He inferred that the result may be a consequence of "infections in early childhood transmitted by unhygienic contact with older siblings". Congruent results have later been reported in several studies (Räsänen et al. 1997, Bodner et al. 1998, Pekkanen et

al. 1999). Corresponding indirect evidence of an atopy-preventing effect of microbes is offered by studies showing reduced risk of atopy in children growing up in a farm environment (Kilpeläinen et al. 2000, Riedler et al. 2001). The same mechanisms may be related to early-life exposure to pets (Nafstad et al. 2001, Remes et al. 2001).

Evidence of the role of commensal flora on Th cell balance is offered by studies evaluating gut microflora and atopy. Sudo et al. (1997) showed in germ-free mice that intestinal bacterial flora was obligatory for converting the early Th2 cell dominance to a Th1-type response and for the development of oral tolerance. Human studies have demonstrated significant differences in gut microflora between atopic and non-atopic subjects (Björksten et al. 2001, Kalliomäki et al. 2001a). Early manipulation of gut microflora via oral administration of probiotic bacteria has been shown to provide some protection against atopic eczema (Kalliomäki et al. 2001b, 2003). Antibiotic use during infancy may, by contrast, disturb gut flora, thereby preventing postnatal Th1 cell maturation (Oyama et al. 2001, McKeever et al. 2002). These results support the theory that factors interfering with colonization may have a role in the development of atopy.

Pathogenic microbes obviously also affect atopic sensitization. Several specific infections (Shaheen et al. 1996, Matricardi et al. 1997, von Mutius et al. 2000) have been shown to be related to a reduced risk of atopy. The role of common viral respiratory infections in atopic sensitization is, however, more complicated. Early viral respiratory infections have been found in several studies to increase the risk of asthma or asthma-like respiratory symptoms (Nafstad et al. 2000). This is particularly the case with infections induced by respiratory syncytial viruses (Sigurs et al. 1995, Stein et al. 1999) and rhinoviruses (Kotaniemi et al. 2003), but their role in atopic sensitization is controversial. While some studies have found an association between respiratory syncytial virus infection and atopic sensitization (Sigurs et al. 1995), others have not (Stein et al. 1999). Numerous mechanisms likely underlie the link between microbes and atopy. Possibilities include a bidirectional interaction, an atopic state influencing airway responses to viral infection, and a viral infection influencing atopic sensitization.

The role of passive smoking in sensitization also remains obscure. Halken et al. (1995) concluded in their review that passive smoking causes an increased risk of obstructive respiratory disease as well as an increased risk of developing sensitization to specific allergens. In a relatively new prospective study, both prenatal and postnatal passive smoking was reported to have an adjuvant effect on allergic sensitization (Kulig

et al. 1999b). Strachan and Cook (1998), by contrast, reported that parental smoking is unlikely to increase the risk of allergic sensitization. They had excluded from their analyses subjects who had symptoms of asthma. Thus, passive smoking obviously increases the risk of obstructive respiratory disease and may also increase the risk of atopic sensitization. Tobacco smoke-related mechanisms causing sensitization probably differ from those of inhalant allergens.

Exposure in early life to inhalant allergens such as house dust mites and pollen has been shown to be an important determinant of subsequent development of asthma (Sporik et al. 1990, Arshad et al. 1993), but the role played by exposure to pets is, at present, unclear (Wahn et al. 1997, Lindfors et al. 1999, Linneberg et al. 2001, 2003, Nafstad et al. 2001, Remes et al. 2001). The significance of exposure to pets probably depends on timing of exposure and co-existence of other risk factors, especially microbes.

Epidemiology of premature birth

Preterm birth is defined as birth before 37 weeks' gestation. The proportion of preterm infants of all newborns in Finland during the last decade has been 5-6%, VLBW infants constituting approximately 15% of all preterm births (National Birth Register 1991-2001). Preterm birth is multifactorial and difficult to predict despite several known risk factors (i.e. demographic, behavioural, maternal pre-pregnancy- and pregnancy-related factors) (Kliegman and Das 2002); the cause of 30-50% of preterm births remains unidentified (Hakala and Ylikorkala 1989, Slattery and Morrison 2002). The most common risk factors for preterm birth in Finland are multiple gestation, pre-eclampsia, hypertension, fetal malformations and growth retardation, previous preterm delivery, preterm uterine contractions, smoking, bleeding during early pregnancy, inadequate prenatal care and unmarried status (Hakala 1987, Hartikainen-Sorri and Sorri 1989).

Approximately 20-30% of preterm births are due to medical or obstetric complications of pregnancy (e.g. pre-eclampsia, placental problems), where preterm delivery is indicated for the well-being of the mother or foetus, one-third to preterm uterine contractions and one-third to preterm premature rupture of membranes. The most common causes for premature rupture of membranes are multiple pregnancy, polyhydramnion and infection. Infection is estimated to be related to 20-40% of

premature births. Cervix insufficiency has been linked to 8-15% of cases (Kekki and Paavonen 2003).

NORMAL DEVELOPMENT OF ORGANS AND IMMUNE MECHANISMS INVOLVED IN ATOPIC DISEASES

Foetal development of organs

Immune system

Development of the immune system occurs early in life. Lymphocytes, antigen-presenting cells (APCs) and phagocytic cells all derive from pluripotent stem cells present in the human yolk sac at 21 days of gestation. Starting in the fifth week, the liver, spleen, thymus and bone marrow take over production of these cells (Hayward 1998).

By 22-23 weeks of gestation, mature polymorphonuclear neutrophils of the innate immune system are few in number, approximately 2% of those measured in the cord blood of neonates born at term (Ohls et al. 1995). At birth, in both term and preterm infants, these cells, as well as mononuclear phagocytic cells and natural killer cells, are functionally competent in normal circumstances, but under stress their functional capacity is impaired, leading to an increased susceptibility to infections during the neonatal period. The deficit is more pronounced in preterm infants (Kapur et al. 2002).

Differentiation and functional maturity of T cells, which appear in week 8 of fetal life (Hayward 1998), is presumed to be complete by 18-20 weeks of gestation (Hanson et al. 1997). At birth, however, T cells still have low helper, suppressor and cytotoxic functions as well as diminished cytokine production (Hanson et al. 1996, 1997), increasing the susceptibility to intracellular and parasite infections (Hanson et al. 1997). Immunodeficient status due to functional immaturity of T cells persists at least until the age of 4-5 years (Pirenne et al. 1992).

Foetal B cells have been found in in vitro studies to have the ability to produce IgM by gestational week 8, and IgG slightly later (Hanson et al. 1997, Hayward 1998, Kapur et al. 2002). IgG antibodies recognized in foetuses earlier, even at 5-6 weeks´ gestation, are of maternal origin and have been transported across the placenta; IgG is the only immunoglobulin capable of crossing the placenta (Hanson et al. 1997). IgA has

been found in foetal gut preparations derived from aborted foetuses at 13 weeks´ gestation (Hayward 1998), but other studies have demonstrated IgA synthesis at about 30 weeks' gestation (Kapur et al. 2002). IgE antibodies have been measured in foetal blood at around 22 weeks´ gestation, and from then on the allergen-specific foetal proliferative responses have been found to correlate with increasing gestational age (Jones et al. 1996). Although the foetus is capable of producing different antibodies, the total antibody-producing capacity at birth even at term is still significantly lower than in adults.

At the time of birth, most of the fetal circulating antibodies are IgG antibodies of maternal origin. The IgG concentration decreases postnatally because of catabolism of maternal IgG and reaches a nadir, a physiological hypogammaglobulinemia, at about 3-4 months of age. Adult IgG levels are reached by 4-6 years (Kapur et al. 2002), depending on the subclass in question. IgG1 and IgG3 subclasses have been shown to reach adult levels faster than IgG2 and IgG4. The circulating IgM level at birth is only 5-20% of the adult value (Hayward 1998, Kapur et al. 2002), reaching the adult level by 1-2 years of age (Kapur et al. 2002). The adult level of IgA in serum is attained around puberty (MacDonald 1996, Kapur et al. 2002).

Maturation of the immune system is a continuous process, starting in the first weeks of foetal life and extending into adulthood (Schultz et al. 2000). The first two years of postnatal life are considered to form a sensitive period during which the immunological priming of the Th cells takes place (Yabuhara et al. 1997, Macaubas et al. 1999), influenced by several intrinsic and extrinsic factors.

Gut

Development of the intestinal system also occurs early, the major morphological events taking place in the first 2 months after conception (MacDonald et al. 1996). Functional capacity, by contrast, continues to mature throughout the foetal period, reaching far into the postnatal phase (Bates and Balistreri 2002).

Peyer´s patches and the first lymphocytes in lamina propria and between epithelial cells (components of the gut-associated lymphoid tissue) appear at 11 weeks´ gestation (Bates and Balistreri 2002). M cells, specialized antigen uptake cells overlying Peyer's patches, have been observed at 17 weeks. The first T cells in the intestine appear at around week 19, probably having migrated from the thymus. B cells appear earlier and are relatively mature in the small intestine by 16 weeks´ gestation, but remain very few

in number even at the time of birth around 40 weeks' gestation (MacDonald et al. 1996). Postnatally, IgA and IgM production increase rapidly in the intestinal lamina propria (Perkkiö and Savilahti 1980, MacDonald et al. 1996, Hanson 1997), with IgM synthesis dominating (Perkkiö and Savilahti 1980). Within 3 months, however, the predominant immunoglobulin in lamina propria is IgA, reaching adult levels by 2 years (Savilahti 1972).

The developmental state of the gut is also reflected in intestinal permeability. At birth, preterm infants have a higher permeability to proteins (Roberton et al. 1982, Müller et al. 1986, Axelsson et al. 1989, Kuitunen et al. 1994) as well as to disaccharides (van Elburg et al. 2003) than infants born at term. Protein permeability decreases with increasing maturity (Axelsson et al. 1989, Kuitunen et al. 1994). The effect of the increased intestinal permeability on priming of the immune responses of preterm infants is still unknown.

Lungs

Lung development is divided into three periods: embryonic, foetal and postnatal. The foetal period consists of four stages: pseudoglandular, canalicular, saccular and alveolar (Jobe 2002). Throughout the process, these periods and stages partly overlap.

The first lung bud appears at 4 weeks´ gestation. In the embryonic period (from 4 to 6 weeks), the proximal airways and pulmonary artery are formed. During the first part of the foetal period, in pseudoglandular stage (from 7 to 16-17 weeks), airway branching results in formation of bronchioles. Pulmonary arteries and veins grow in tandem with the airways. The acini are built at the canalicular stage (from 16-17 to 25-27 weeks). During this stage epithelial cells lining the airways also differentiate, surfactant synthesis starts and the capillary network between airspaces is formed. In the saccular stage (25-28 to 35-40 weeks), the gas-exchange sites expand and final branching of airways takes place. In the alveolar stage, from 36-40 weeks' gestation to 1-3 years postnatally, thin-walled alveoli are finished and multiplied (Hansen and Corbet 1998a, Jobe 2002). Lung growth continues thereafter. The microvascular network matures until early preschool age. The rate of lung maturation is determined multifactorially, by genetic factors, cytokines and hormonal and pharmacological factors (Jobe 2002).

Surfactant, functionally the most important biochemical component in the lungs, emerges at about 24 weeks´ gestation, with its synthesis increasing progressively

thereafter. The appearance of surfactant in airspaces is enhanced by several factors (labour, delivery and breathing, corticosteroids, thyroid hormones and drugs such as theophyllin), enabling the infant's survival after premature birth (Jobe 2002).

Most preterm infants begin breathing with structurally and functionally very immature lungs, which leads frequently to early respiratory distress syndrome (RDS). In RDS, peripheral air spaces, relatively few in number, collapse easily, after which the more proximal respiratory bronchioles become overdistended and are covered by necrotic epithelium and hyaline membranes primarily because of the insufficient surfactant system. This often results in a difficult cascade of respiratory complications (Hansen and Corbet 1998a).

Foetal and postnatal T helper cell balance

Foetomaternal immune balance

During pregnancy maternal cell-mediated immunity is transiently depressed (Lin et al. 1993, Sabahi et al. 1995), and Th2-deviated immune balance dominates in the maternal and foetoplacental immune system, which is considered to be fundamental to the maintenance of a successful pregnancy (Wegmann et al. 1993). Wegmann et al. (1993) showed this in their murine studies; decidual cells spontaneously released interleukin-4 (IL-4), IL-5 and IL-10 in multifold amounts compared with stimulated spleen cells, but interferon- γ (IFN-γ) in only very low quantities. The human foetoplacental unit has also been found to produce significant amounts of cytokines IL-4 (Jones et al. 1995), IL-10 (Roth et al. 1996) and IL-13 (Williams et al. 2000). With these cytokines, the foetoplacental unit is assumed to redirect the maternal immunity towards Th2-dominated immune responses (Lin et al. 1993). Besides their role in maintenance of pregnancy, Th2-type cytokines promote foetal development and growth.

Th1-type cytokines, by contrast, are associated with harmful cytotoxic effects on pregnancy (Piccinni and Romagnani 1996), similar to their influence in allograft rejection. Analyses of aborted foetal tissues support this theory. The decidua of women with unexplained recurrent abortion have been found to produce significantly lower concentrations of IL-4 than clones derived from the decidua of voluntary abortions or of the endometrium of non-pregnant women (Deneys and Bruyere 1997). Thus, the normal

pregnancy-related Th2 cell-dominant balance obviously protects the foetus against rejection (Mellor and Munn 2000, Thellin et al. 2000).

The cytokine pattern during pregnancy is controlled by several hormones. Progesterone affects the differentiation of Th cells into Th2 cells (Piccinni et al. 1995), whereas relaxin, another corpus luteum-derived hormone, mainly promotes the development of Th1-type cells (Piccinni and Romagnani 1996). Prostaglandin E2 is also known to contribute to immune balance by inhibiting IL-2 and IFN-gamma (Betz and Fox 1991), and probably suppressing the activity of natural killer cells (Linnemeyer and Pollack 1993). Prostaglandin E2 is viewed as a general immunosuppressant.

The theoretically possible effect of maternal atopy on the course of pregnancy through Th2 cell-deviated balance has not been closely studied, but some conclusions have been drawn from indirect data. In an epidemiological study, atopic mothers were reported to have on average more children than non-atopic mothers, and thus, a positive association was concluded to be present between maternal atopy and successful pregnancy (Nilsson et al. 1997). However, controversial findings also exist. Sunyer et al. (2001), for instance, found an inverse relationship between maternal atopy and parity.

In contrast to maternal atopy, the association between maternal asthma and preterm birth has been widely studied. Maternal asthma has repeatedly been linked to an adverse pregnancy outcome and an increased risk of preterm labour (Kelly et al. 1995, Kramer et al. 1995, Demissie et al. 1998, Källen et al. 2000, Liu et al. 2001). Kramer et al., however, found no association between metacholine responsiveness and preterm birth, suggesting that non-atopic, non-cholinergic mechanisms may link bronchial and uterine smooth muscle lability. Discrepant findings have also been reported; several studies have observed no association between optimally controlled asthma and preterm labour (Jana et al. 1995, Alexander et al. 1998, Minerbi-Codish et al. 1998). Tan and Thomson (2000) concluded in their review that adverse outcomes of pregnancy (e.g. preterm labour) can be attributed to chronically poor asthma control, whereas women with well-controlled asthma during pregnancy have outcomes similar to their non-asthmatic counterparts.

Transplacental sensitization

Neonatal T cells are capable of responding to specific allergens at birth, indicating prenatal transplacental sensitization (Kondo et al. 1992, Piccinni et al. 1993, Warner et

al. 1994, Holt et al. 1995, Jones et al. 1996, Furuhashi et al. 1997, Prescott et al. 1998, 1999). Several groups have shown this by measuring low-level lymphoproliferative responses from stimulated cord blood mononuclear cells (CBMC) to both inhalant and food allergens (Kondo et al. 1992, Piccinni et al. 1993, Warner et al. 1994, Holt et al. 1995, Jones et al. 1996, Miles et al. 1996). Based on the common foetal Th cell balance, these neonatal allergen-specific T cell responses represent the Th2 cell-derived cytokine profile. Less is known about the timing of sensitization. Hauer et al. (2003) found that spontaneous cytokine-secreting cells were virtually absent in cord blood of infants under 34 weeks' gestation, whereas Jones et al. (1996) have reported signs of proliferative cell responses at 22 weeks' gestation.

The origin of prenatal antigen-specific T cells has occasionally been impugned. Prescott et al. (1999) have, however, confirmed that the responding and proliferating cord blood cells were of foetal and not maternal origin by performing DNA analysis. Moreover, most infants showed no response to tetanus toxoid at birth (Holt et al. 1995, Prescott et al. 1998), which also indicates foetal origin of the cells.

The role of transplacental sensitization in later atopic predisposition remains ambiguous. While some groups have reported that it predicts development of later atopic sensitization (Kondo et al. 1992, 1998, Warner et al. 1994, Miles et al. 1996), it has also been proposed to be related to normal adaptation to wide postnatal antigen exposure (Prescott et al. 1998).

Postnatal conversion of T helper cell balance

At birth, virtually all neonates express low-level Th2-skewed cytokine responses, similar to those in atopic adults (Prescott et al. 1998, 1999). However, from the early postnatal period onwards, maturation of the immune system leads to a rapid suppression of foetal Th2-type responses. Levels of IL-4 and IL-13 responses gradually fall, while IFN-gamma production increases (Prescott et al. 1999). The process is modified by genetic and environmental factors. In non-atopic infants, the Th1/Th2 cell balance is normally converted into Th1 predominance during the first year of life (Prescott et al. 1999, Macaubas et al. 2000).

The mode of postnatal suppression of Th2 responses diverges depending on the nature of the allergen. Responses to food and aeroallergens have different age-related changes (Yabuhara et al. 1997, Prescott et al. 1999). Proliferative responses to ovalbumin have been found to decline rapidly after birth and to be virtually absent by

12 months, whereas T cell reactivity to aeroallergens after birth increases progressively (Yabuhara 1997), the cytokine pattern gradually (in non-atopics) polarizing towards Th1-type responses (Macaubas et al. 2000). The divergent age-related patterns of change in these neonatal proliferative responses against food and inhalant allergens have been demonstrated to have no significant association with family history of atopy or expression of atopic signs (Prescott et al. 1999). The mechanisms underlying the regulation of lymphoproliferative postnatal changes are yet to be identified (Yabuhara et al. 1997).

Normal patterns of immune responses to food and inhalant antigens

All environmental antigens entering the host induce a set of physiological immune responses, either systemic priming, systemic tolerance (specific immunological unresponsiveness against harmless antigens) and/or induction of local secretory IgA responses in the absence of systemic immune responses (Strobel and Mowat 1998). Food antigens induce in most healthy children and adults antigen-specific IgG (mainly subclasses of IgG1 and IgG4), IgA, IgM and IgE antibodies (Husby 2000), with levels in healthy children gradually declining with increasing age (Barnes et al. 1995, Yabuhara et al. 1997, Jenmalm and Björksten 1998). The earlier the foreign antigen is introduced, the higher the antibody level rises (Tainio et al. 1988, Keller et al. 1996, Oldaeus et al. 1999) and the longer the elevated titre remains (Jenmalm and Björksten 1998), indicating the influence of timing of exposure.

Oral tolerance, physiological unresponsiveness against ingested antigens, starts to grow gradually after birth. In the mouse, it develops via multiple, complex pathways; in man the exact mechanism remains unclear. Development of tolerance is always preceded by activation of antigen-specific T cells, after which tolerance is suggested to occur through anergy, deletion of T cells and/or suppression of T cell responses by regulatory T cells and their products, IL-10 and transforming growth factor-β (TGF-β) (Garside and Mowat 2001); both Th1- and Th2-mediated responses are down-regulated (Garside et al. 1995).

The predominant mechanism of tolerance depends on circumstances of antigen exposure. The nature, site, dose and timing of the antigen employed are thought to have an influence (Garside and Mowat 2001). High doses of antigen have been proposed to

promote clonal deletion, several dosings anergy of reactive T cells and lower doses activation of regulatory T cells which suppress immune responses (Mayer 2000). Mechanisms may also co-exist.

Single feeds have been shown to inhibit IgM, IgG and IgE antibody responses as well as cell-mediated responses, IgE responses being particularly susceptible to induction of tolerance (Strobel and Mowat 1998). Decline in serum IgG and IgE antibody responses together with diminished T cell responses are considered the hallmarks of oral tolerance (Kato et al. 2001). In food allergies, development of tolerance has failed (Strobel and Mowat 1998).

Several host-dependent factors (genetic background, age, maturation, hormonal status, gut flora, amount of antigen absorbed) are also suggested to influence the development of tolerance (Mayer 2000, Garside and Mowat 2001). A clear relationship between the amount of antigen absorbed and induction of tolerance has not been demonstrated. The role of immaturity of the host also remains unclear; in rodent studies, it has been found to be related to a decreased ability to induce tolerance (Strobel and Ferguson 1984, Miller et al. 1994). However, Takahashi et al. (1995) showed that human cord blood T cells were highly susceptible to tolerance induction.

Allergen-specific IgG antibodies to seasonal antigens can also be detected in early life (in 25% of subjects under 1 year of age), but in contrast to food antibodies, the concentrations are found to steadily increase with age, with 40-50% of children having measurable levels of specific IgG antibodies by age 5 (Rowntree et al. 1985). Yabuhara et al. (1997) have measured in over 90% of adults moderate to high levels of lymphoproliferative responses to house dust mite compared with concomitant low frequency and low intensity responses to ovalbumin. Allergen-specific inhalant IgE antibodies are detected in less than 5% of subjects under 1 year of age and in about 20% by 5 years of age. High levels are found to persist especially in those who develop clinical allergic symptoms (Rowntree et al. 1985, Hattevig et al. 1993).

Divergent patterns of normal immune responses to different environmental antigens apply to qualitatively different types of control mechanisms; immune deviation (suppression) being dominant for nanogram-level exposure of inhalant allergens (leading to low zone tolerance) in contrast to T cell deletion or anergy for milligram-level to gram-level exposure of dietary allergens (leading to high zone tolerance) (Holt 1998).

DEVELOPMENT AND MECHANISMS OF THE IMMUNE SYSTEM IN ATOPIC CHILDREN

Characteristics of T helper cell balance in atopic individuals at and after birth

Differences in immunological status of atopic and non-atopic individuals can be seen at birth. Piccinni et al. (1996) found that CBMC of newborns with atopic parents exhibited an enhanced ability to produce Th2 cytokines (IL-4 and IL-5) compared with cells of newborns with non-atopic parents, but the ability to produce IFN- γ did not differ between the groups. In addition, Miles et al. (1996) discovered that infants who were born to atopic parents and/or who developed an allergic disease by the age of one year significantly more often had measurable allergen-specific peripheral blood mononuclear cell (PBMC) proliferative responses at birth than infants who did not develop any allergy or those without a family history of atopy.

Holt et al. (1992) came to a different conclusion. They reported that infants at high genetic risk for atopy had markedly lower IFN-γ- and IL-4-producing capacity than controls at birth. They later specified that in atopics just the Th2 cell cytokine responses (IL-4, IL-6, IL-10, IL-13) were significantly lower at birth associated with defective IFN- γ production, and that the normal postnatal changes in Th cell cytokine profiles failed to occur in a timely fashion, resulting in prolongation of foetal Th2-type responses (Prescott et al. 1999). Williams et al. (2000) reported a congruent result; a low level of cord blood IL-13 was demonstrated to be associated with a later risk of atopy. Defective IFN- γ production at birth in infants at high risk for atopy or in those who later develop atopic disorders has been shown by several groups (Tang et al. 1994, Warner et al. 1994, Liao et al. 1996, Kondo et al. 1998, Macaubas et al. 2000).

The basic defect in the immune system of atopic individuals has therefore been suggested to be a significantly decelerated postnatal maturation of the Th cell balance, and not prenatal sensitization (Prescott et al. 1998, 1999). Atopic infants do not exhibit an adult-type cytokine profile until around the age of 6 years (Macaubas et al. 2000), in contrast to non-atopic infants who attain this profile at 1-2 years. Based on this, the first years of life are considered to form a critical period for the development of Th cell

memory (priming of Th cells) and the allergen-responder phenotype in later life (Yabuhara et al. 1997, Macaubas et al. 1999).

The association between cord blood IgE levels and subsequent predisposition to atopy has also been widely studied. High cord blood IgE levels are highly specific, but their sensitivity to predict atopy has been found to be low (Arshad et al. 1993, Kobayashi et al. 1994, Bergmann et al. 1997, Edenharter et al. 1998).

Immune responses in atopic diseases

According to the current theory, the principal cause of atopy is thought to be related to a disturbed balance in Th cell subsets, followed by inappropriate IgE secretion from B cells. The two main functional subsets, Th1 and Th2 cells, arise from a pluripotent precursor cell termed Th0. Differentiation and activation of Th1 cells, which produce predominantly IL-2, IFN-γ and tumour necrosis factor-beta (TNF-β) (Mosmann et al. 1986, Imboden and Seaman 2001), lead to cell-mediated cytotoxic immune responses, macrophage activation and delayed type hypersensitivity (Imboden and Seaman 2001). Th1 cell-mediated responses are produced especially in bacterial and viral infections (Romagnani 1992). Th2-type cells produce predominantly IL-4, IL-5, IL-6, IL-10 and IL-13 (Huston 1997, Imboden and Seaman 2001) and contribute through activation of B cells and effector cells to protective humoral immune responses in parasite infections. In addition, they participate in allergic reactions (Huston 1997, Imboden and Seaman 2001). Determinants for differentiation of naive Th cells into various successors and different types of immune responses are not fully understood. The process occurs under the influence of a complex interplay of genetic and environmental factors. Such factors as nature and dose of antigen (and adjuvant), site and timing of exposure and other ongoing immune responses are proposed to play a role in these processes (Romagnani 1992). In atopic diseases, the Th cell balance is pathologically skewed to Th2 phenotype responses (Imboden and Seaman 2001).

In allergic sensitization, immune responses are initiated by allergen bounding to HLA class II molecules on APCs. Macrophages, monocytes, dendritic cells and B lymphocytes possess APC properties. APCs process and present the allergen to naive T cells, Th0 cells, through T cell receptors (TCRs) and with the assistance of other surface molecules (especially CD4) on the T cell membranes (Huston 1997, Imboden and

Seaman 2001). After antigen presentation, the Th cells differentiate with a complex cell-cell interaction and under the influence of stimulatory and inhibitory soluble mediators into a subpopulation of Th2 cells. Differentiated Th cells induce via cytokines and interaction of cell membrane molecules the maturation of B cells into antigen-specific, immunoglubulin-producing plasma cells (Huston 1997). A portion of the activated Th cells are transformed into memory T cells. The mature B cells activated by the Th2 cell route produce IgE and IgG4 antibodies (Huston 1997, Imboden and Seaman 2001). IgE antibodies bound to high-affinity cell membrane receptors on effector cells (Costa et al. 1997).

In a re-exposure, allergen molecules react with IgE antibodies on effector cell (mast cells, basophils, eosinophils) receptors. The cells are directly activated and start to secrete mediators of inflammation (histamine, leucotrienes, chemotactic factors, prostaglandins, platelet-activating factor, proteases). In an immediate phase of the allergic reaction, mediators act locally and cause increased vascular permeability, vasodilatation, smooth muscle contraction and mucous gland secretion. In the late phase, cellular inflammation develops; inflammatory cells (neutrophils, eosinophils, mononuclear cells) infiltrate into the tissue in response to chemical mediators (Terr 2001).

PATHOLOGICAL CHANGES IN ASTHMA AND IN NEONATAL CHRONIC LUNG DISEASE

In addition to histochemical inflammation in asthma, specific structural changes develop in the airway walls. This process, referred to as airway remodelling, comprises subepithelial fibrosis, myofibroblast accumulation, airway smooth muscle hyperplasia and hypertrophy, mucous gland and goblet cell hyperplasia and epithelial disruption. The airway remodelling may be a consequence of chronic inflammation, but the precise relationship between the remodelling and inflammatory components in asthma remains unclear (Redington 2000, Jeffery 2001).

Pathogenesis of CLD is obviously multifactorial. Immaturity of the lungs, including a deficient surfactant system, has a primary role, but genetic factors and postnatal treatments and events (exposure to oxygen toxicity, barotraumas/volutrauma,

infections, excessive hydration, nutritional insufficiency) also contribute markedly to the development of the disease (Hansen and Corbet 1998b), resulting in aberrant lung development (Jobe 1999). Postmortem examinations of BPD lungs have revealed massive fibrosis and destruction of alveoli and airways, bronchial smooth muscle hypertrophy, metaplasia of the airway mucosa, loss of pulmonary arterioles and capillaries and muscular hypertrophy of the remaining vessels (Hansen and Corbet 1998b), leading to impaired airway growth in infancy. Histochemically, a persistent neutrophilic inflammation prevails. However, in some studies, eosinophils have also been concluded to participate in the pathogenesis of CLD (Yamamoto et al. 1996, Raghavender and Smith 1997).

The pathogenesis of long-term respiratory symptoms and lung function abnormalities of prematurely born infants at school-age is less well known. Long-term respiratory symptoms are suggested to be mainly due to structural pulmonary changes (Northway et al. 1990, Chan and Silverman 1993), but inflammatory mechanisms obviously also play a role in the process (Pelkonen et al. 1999).

ATOPY IN PREMATURELY BORN CHILDREN

Atopic sensitization and atopic diseases

Previous studies evaluating the relationship between atopy and prematurity have yielded inconsistent results, reporting both positive and inverse associations. Summaries of these studies are presented in Tables 1a-c. The studies were collected by performing an electronic literature search by cross-linking the search words “birth weight”, “gestational age”, “preterm”, “prematurity”, “atopy” and “allergy”.

Preterm birth was earlier thought to be a risk factor for atopic sensitization since immunological and digestive immaturity was assumed to lead to an impaired development of tolerance (Strobel and Ferguson 1984, Arsdhad et al. 1993, Clough 1993). This theory has been supported by population-based epidemiological studies and by some cohort studies of selected groups of children (Table 1a). In these studies, asthma, allergic rhinitis and/or atopic eczema were each found to be related to low gestational age or to low birth weight. Food allergen avoidance and aeroallergen elimination programmes, which have previously been recommended as the primary

means for prevention against sensitization (Hide et al. 1996), were based on this theory of impaired development of tolerance.

Several other studies have, however, found no significant association between atopy and gestational age or low birth weight (Table 1b). These results lead to the assumption of equal functional capacity to develop tolerance or sensitization irrespective of immunological maturity stage during the neonatal period.

David and Ewing (1988) were the first to report an inverse association between prematurity and atopy; they noted the exceptionally low number of subjects born preterm among 443 children hospitalized because of atopic dermatitis. Klebanoff and Berendes (1988) responded that in their large birth cohort of 144 793 subjects the difference in eczema prevalence between subjects born preterm and full-term was minimal and not significant. A few cohort studies and several epidemiological studies have, however, subsequently confirmed the findings of David and Ewing (Table 1c).

General antibody production capacity of preterm infants has been evaluated by analyzing their antigen-specific vaccine responses. Preterm infants have been reported to yield adequate protective antibody responses against vaccine antigens (Faldella et al. 1998, Khalak et al. 1998, Schloesser et al. 1999, Thayyil-Sudhan et al. 1999, Kirmani et al. 2002), although the antibody concentrations were significantly lower than in term infants (Faldella et al. 1998, Schloesser et al. 1999, Kirmani et al. 2002).

Atopy in relation to wheezing, asthma and lung function

Long-term respiratory symptoms and lung function in prematurely born children have been studied extensively. Children born preterm, as compared to those born full-term, are known to have more long-lasting asthma-type respiratory problems (Chan et al. 1989a, Kitchen et al. 1992, Frischer et al. 1993, Rona et al. 1993, Elder et al. 1996, McLeod et al. 1996, Svanes et al. 1998) and reduced lung function (Chan et al. 1989b, Northway et al. 1990, Rona et al. 1993, Parat et al. 1995, Hakulinen et al. 1996, McLeod et al. 1996, Pelkonen et al. 1997, Jacob et al. 1998, Kennedy et al. 2000). Those who have had BPD/CLD in particular have an increased risk of long-term respiratory problems (Bader et al. 1987, Northway et al. 1990, Koumbourlis et al. 1996, Giacoia et al. 1997, Gross et al. 1998, Jacob et al. 1998). Prematurity is thus commonly considered a risk factor for childhood asthma. However, this conclusion has not been

Table 1a. Cohort studies of prematurely born children and/or low birth weight children and population-based studies that evaluated the association between atopic findings and perinatal factors and found **prematurity and/or low birth weight to increase the risk of atopy**.

Authors (Year) Country	Objective of study	Study cohort/ groups	Methods/ data collection	Age of children	Outcome measures	Results/ conclusions
Lucas et al. (1990) United Kingdom	To study the effect of early diet and incidence of allergic reactions in preterm infants	Follow-up of 777 children with BW <1850 g	Questionnaire Physical examination Exclusion-challenge test	18 mo	History of AD and wheezing Challenge-confirmed food sensitivity	Incidence of eczema and wheezing higher in preterms than in normal population
Forster et al. (1990) Germany	To study the effect of gestational age, nutrition and social class on atopic symptoms	Follow-up of 318 newborns treated in the ward (137 born preterm, <38 wks)	Perinatal data on hospital records Questionnaire (phone calls to check questionnaire data)	18 mo	History of AD, asthma and hay fever	Incidence of atopic symptoms overall higher in children born preterm ($p<0.01$), ($p<0.001$ for difference in incidence of AD)
Kuehr et al. (1992) Germany, Austria	To study early childhood risk factors of atopic sensitization	Cohort of 1470 schoolchildren	Questionnaire SPT	6 - 8 y	Positive SPT	Gestational age <37 wks is a risk factor for aeroallergen sensitization (OR 1.9, 95% CI 1.1-3.2)
Arshad et al. (1993) United Kingdom	To determine the effect of genetic and environmental factors on prevalence of allergic disorders	Prospective follow-up of a birth cohort of 1174 children	Perinatal data on hospital records Questionnaire SPT (n=436) Cord blood IgE	2 y	Doctor-diagnosed asthma, AD, rhinitis and food allergy Positive SPT	BW <2500 g but not prematurity increased the risk of asthma ($p<0.01$) and positive SPT ($p<0.01$) but not the risk of AD or rhinitis
Stazi et al. (2002) Italy	To study the influence of early life events on IgE-mediated allergy	Population-based sample of 201 children	Questionnaire Interview SPT	3 mo - 5 y	History of AD, allergic rhinitis and asthma Positive SPT	Prematurity (<37 wks) and low BW (<2500 g) increased the risk of rhinitis but were not associated with SPT-positivity

AD = atopic dermatitis, BW = birth weight, SPT = skin prick test, wks = gestational weeks

Table 1b. Cohort studies of prematurely born children and/or low birth weight children and population-based studies that evaluated the association between atopic findings and perinatal factors and found **no significant association between prematurity and/or low birth weight and atopy**.

Authors (Year) Country	Objective of study	Study cohort/ groups	Methods/ data collection	Age of children	Outcome measures	Results/ conclusions
de Martino et al. (1989) Italy	To evaluate atopic diseases in preterm infants	80 preterm (27 - 38 wks), 80 full-term infants	Questionnaire SPT	9 - 24 mo	History of AD, urticaria and wheezing Positive SPT	No significant difference in frequencies of SPT reactions or in atopic diseases between infants born preterm and full-term infants
Savilahti et al. (1993) Finland	To evaluate the effect of early feeding and appearance of allergic sensitization on infants born preterm	69 preterm infants (31 - 36 wks)	Questionnaire SPT Serum total and cow milk-specific IgE, IgG, IgA, IgM	11 y	History of allergic rhinitis, urticaria, AD and wheezing Positive SPT	Cumulative incidence of allergic symptoms similar to that reported in unselected series (prolonged exclusive breast feeding increased the risk of atopy)
Sears et al. (1996) Canada	To study risk factors for development of childhood atopy	Follow-up of birth cohort of 1037 children	Questionnaire SPT (at 13 y) Spirometry with metacholine challenge	18 y	History of asthma and hay fever Positive SPT BHR	No association between BW and atopy (defined as positive SPT), asthma or BHR
Butland et al. (1997) United Kingdom	To investigate whether changes in perinatal and social factors explain the increase in prevalence of hay fever and AD	2 birth cohorts, 11 195 children born 1958, and 9387 born 1970	Perinatal data collected by midwives at birth Parental interview at 5 or 7 y, and at 16 y	16 y	History of current hay fever and AD	Neither hay fever nor AD was associated with BW (BW did not explain the increase over a 16-y period in prevalence of hay fever or AD)
Fergusson et al. (1997) New Zealand	To study linkage between perinatal factors and the risk of atopic conditions	Longitudinal follow-up of a birth cohort of 891 children	Structured interview Data from hospital records, general practi-tioner's notes and diary kept by parents	16 y	Number of medical consultations because of asthma, allergic rhinitis, AD or other atopic illnesses	Neither gestational age nor BW were related to asthma, AD or allergic rhinitis (large head circumference was linked to an increased risk of asthma)

Gregory et al. (1999) United Kingdom/ New Zealand	To examine the relation between antropometric measurements at birth and asthma and incidence of atopy	Part (239 children) of the Southmapton asthma genetic study	Asthma questionnaire Data from hospital records SPT S-IgE	6 - 26 y	Treated asthma Current wheezing Positive SPT Elevated S-IgE level	No association between gestational age or BW and asthma, S-IgE level or SPT reactions (large head circum ference was linked to high IgE level)
Leadbitter et al. (1999) New Zealand	To assess the relationship between foetal growth and atopy and asthma	Birth cohort of 734 subjects	Perinatal data on hospital records Questionnaire SPT and S-IgE Metacholine challenge	13 y	History of asthma, wheezing, hay fever and AD Positive SPT BHR	BW <3 kg decreased the risk of asthma, but had no association with SPT reactions, S-IgE level, hay fever or AD (head circumference was associated with S-IgE level)
Steffensen et al. (2000) Denmark	To evaluate the association between foetal growth and AD and asthma	4795 male conscripts	Registry data on male conscripts Medical Birth Registry data Medical records	18 y	History of asthma and AD	No significant association between gestational age and asthma or AD
Mai et al. (2003) Sweden	To assess the relationship between VLBW and asthma, lung function and atopy	Prospective follow-up of 74 VLBW children and 64 term children	Questionnaire SPT, IL-4, IL-5, IFN-γ Spirometry with hypertonic saline provocation test	12 y	History of asthma, allergic rhino-conjunctivitis and AD Positive SPT	VLBW was unrelated to atopic symptoms, SPT reactions or cytokine levels, but history of asthma was more frequent in VLBW children

AD = atopic dermatitis, BHR = bronchial hyperresponsiveness, BW = birth weight, S-IgE = serum total IgE, SPT = skin prick test, VLBW = very low birth weight, wks = gestational weeks

Table 1c. Cohort studies of prematurely born children and/or low birth weight children and population-based studies that evaluated the association between atopic findings and perinatal factors and found **prematurity and/or low birth weight to decrease to the risk of atopy**.

Authors (Year) Country	Objective of study	Study cohort/ groups	Methods/ data collection	Age of children	Outcome measures	Results/ conclusions
David and Ewing (1988) United Kingdom	To study the proportion of preterms (<37 wks) among children with atopic eczema	443 children with AD referred to the ward of child health	Regional Health Authority Registry data Questionnaire	-	Doctor-diagnosed AD	Among children with AD, a significantly lower number of subjects born preterm (p=0.03) compared with 81 038 control children (3% vs 6%)
Olesen et al. (1997) Denmark	To study the association between birth factors and development of AD	Birth cohort of 7862 children	Registry data Data on children in dermatology and pediatric wards with diagnosed AD	5 - 8 y	Doctor-diagnosed AD	AD was associated with high gestational age (p=0.02) and with ”heavy-for-date” BW
Bråbäck and Hedberg (1998) Sweden	To study the relationship between perinatal risk factors and allergic rhinitis and asthma	Cohort of 149 398 male conscripts	Linkage of data on Medical Birth Register and Military Service Register	17 - 20 y	Doctor-diagnosed current asthma and allergic rhinitis	Prematurity and low BW protected from allergic rhinitis (<33 wks, 33-36 wks, >36 wks; 11.6%, 13.2%, 15.2%, respectively, p=0.0001) Low BW was related to asthma
Buhrer et al. (1999) Germany	To investigate the 1-y prevalence of atopic eczema in VLBW infants	331 VLBW infants (<1500g) and 455 term controls	Physical examination Questionnaire	1 y	Current eczema History of eczema	VLBW infants had less current eczema (2% vs 4%, p=0.045) and less history of eczema (1.5% vs 4.6%, p=0.016) than healthy controls
Hikino et al. (2001) Japan	To study the prevalence of food allergy and AD in low BW infants	21 766 infants (aged 18mo) and 4378 children (aged 3 y) from well-baby check-ups	Questionnaire Clinical examination by paediatrician	18 mo 3 y	History of doctor-diagnosed food allergy Current AD	Food allergy (p<0.001) and AD (p<0.01) at 18 mo less frequent in children with low BW (<2500 g) (trend was related to gestational age)

Pekkanen et al. (2001) Finland	To study the association of atopy and asthma with pre- and perinatal characteristics	Prospective birth cohort of 5195 subjects	Questionnaire SPT	31 y	Doctor-diagnosed asthma Positive SPT	Risk of atopy (defined as positive SPT) increased with gestational age ≥35 wks (p for trend 0.002), but not with BW No association for asthma
Räsänen et al. (2001) Finland	To study the effect of perinatal factors on the risk of hay fever	Five birth cohorts of twins, 2550 families and 4722 adolescents	Questionnaire	16 y	Parent-reported and doctor-diagnosed hay fever	Risk of hay fever increased with increasing BW (p for trend 0.048) and gestational age (p for trend 0.04), especially in those with no parental history of hay fever
Kerkhof et al. (2003) Netherlands	To study the association between AD and birth characteristics and environmental factors	Part of the birth cohort of the PIAMA study: 304 children of allergic mothers	Questionnaire Physical examination	1 y	Doctor-diagnosed AD	Gestational age was not associated with AD, but BW >4000 g increased the risk of AD compared with weight of 3000-4000 g (OR 2.4, 95% CI 1.1-5.1)

AD = atopic dermatitis, BW = birth weight, PIAMA = Prevention and Incidence of Asthma and Mite Allergy, SPT = skin prick test, VLBW =very low birth weight, wks = gestational weeks

confirmed by all studies. A recent Finnish twin study and a study from New Zealand found no association between gestational age and doctor-diagnosed asthma in adolescents (Leadbitter et al. 1999, Räsänen et al. 2000).

In a few studies concentrating on long-term respiratory morbidity of preterm children (Chan et al. 1989a, Northway et al. 1990, von Mutius et al. 1993, Pelkonen et al. 1997), the incidence of atopy was also examined, but the relationship of long-term respiratory findings to atopy was not analysed. Pelkonen et al. (1997) analysed the frequency of atopy in preterm infants with BPD and in those without BPD (17% vs. 29% were defined as atopic, respectively). Von Mutius et al (1993) reported that premature girls given ventilatory support had atopy less frequently than term girls or those preterm girls who had not been mechanically ventilated.

Atopic heredity has been found in some studies to predispose preterm infants to neonatal respiratory morbidity (Bertrand et al. 1985, Lucas et al. 1990, von Mutius et al. 1993, Elder et al. 1996), but discrepant results have also been presented; several studies have reported no difference in family history of atopy or asthma between preterm infants with and without neonatal respiratory problems (Chan et al. 1989a, Hakulinen et al. 1990, Northway et al. 1990, Hagan et al. 1995, de Winter et al. 1995, Pelkonen et al. 1997). Thus, the role of atopic heredity in long-term respiratory problems of preterm children remains unestablished.

In conclusion, previous studies evaluating the association between atopy and prematurity have failed to establish a consistent result. Thus, further research is needed to determine the long-term effects of immaturity on the immune balance of the child. The possible association between maternal predisposition to atopy and preterm birth also warrants examination. Results of epidemiological studies shed light on the risk factors and protective factors of atopy, which can then be applied to planning of prevention.

AIMS OF THE STUDY

The main objective of this study was to evaluate the association between prematurity and atopy. Specific objectives were:

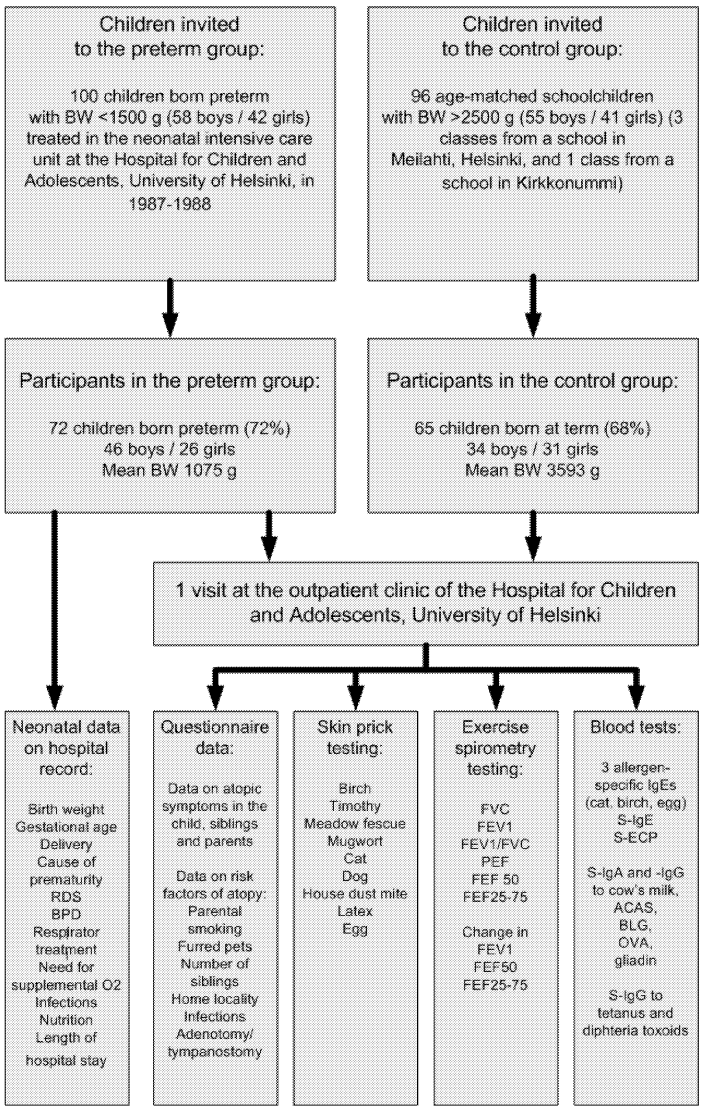
1. To evaluate whether prematurity at birth is associated with a risk of later atopic sensitization of the child by defining prevalence of atopy in children born preterm at the age of 10 years and comparing that with the corresponding prevalence in a control group of children born at term (I).

2. To assess the role of atopy in wheezing, asthma, and lung function in children born preterm compared with children born full-term (II).

3. To compare immune responses to early-introduced food antigens at the age of 10 years of children born preterm with those of children born full-term (III).

4. To evaluate whether maternal atopy is related to preterm birth (IV).

Figure 1. Study design (I, II, III) for evaluation of atopic manifestations in children born preterm.



ACAS = α-casein
BLG = β-lactoglobulin
BPD = bronchopulmonary dysplasia
BW = birth weight
FEF25-75 = forced mid-expiratory flow of FVC
FEF50 = forced expiratory flow after 50% of vital capacity has been exhaled
FEV1 = forced expiratory volume in one second
FVC = forced vital capacity
House dust mite = Dermatophagoides pteronyssinus
OVA = ovalbumin
RDS = respiratory distress syndrome

SUBJECTS AND METHODS

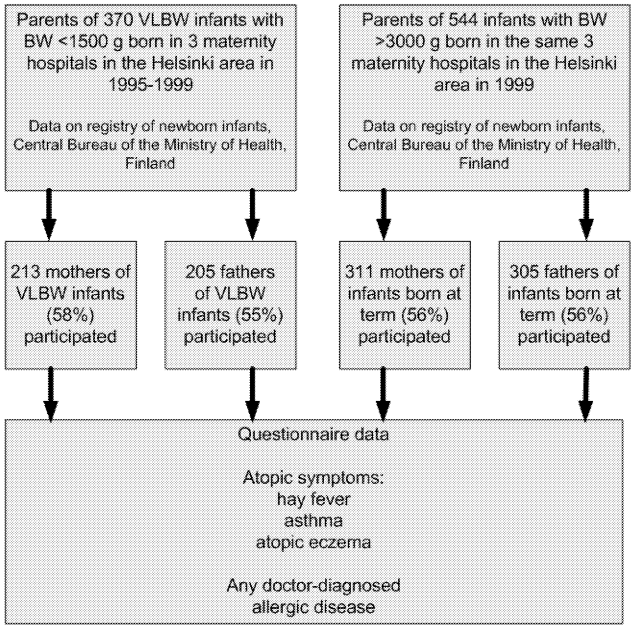
STUDY DESIGN

The relationship between atopy and prematurity was examined from two perspectives:

Immunological and respiratory status of children born preterm were evaluated and compared with those of children born at term, with special reference to atopy (I, II, III). Data on atopic findings, respiratory symptoms and lung function, and immunological responses to early-introduced food antigens were recorded in these two groups of children during one visit at the outpatient clinic of the Hospital for Children and Adolescents (Figure 1).

The association between maternal atopy and premature birth was evaluated by collecting atopy data on two groups of parents by questionnaire (IV). The groups consisted of parents of VLBW infants and parents of infants born at term (Figure 2).

Figure 2. Study design (IV) for evaluation of atopic diseases in the mother and father of VLBW infants.



BW = birth weight
VLBW = very low birth weight

SUBJECTS

Groups of children (I, II, III)

One hundred consecutive very prematurely born children (58 boys, 42 girls) with birth weights of less than 1501 grams, who had been treated in 1987-1988 in the neonatal intensive care unit at the Hospital for Children and Adolescents, University of Helsinki, were invited to participate in the study at the age of 10 years.

The control group consisted of 96 unselected, age-matched schoolchildren (55 boys, 41 girls) from two schools in the Helsinki region (three classes from an urban school in Meilahti and one from a rural school in Kirkkonummi). All of the children from the classes, whose birth weight was over 2500 grams, were invited.

In the preterm group, 72 children (46 boys and 26 girls; 72%) participated and in the full-term group 65 (34 boys and 31 girls; 68%) (Table 2). The difference between the groups in participation was not significant (p=0.513).

Table 2. Characteristics of the children.

	Preterm group (n=72)	Full-term group (n=65)
Male/female	46/26	34/31
Mean birth weight, g (SD, range)	1075 (271, 550-1500)	3593 (470, 2540-5200)*
Mean age, y (SD, range)	10.1 (0.3, 9.6-10.8)	10.1 (0.4, 9.4-10.9)
Mean weight, kg (SD, range)	30.3 (6.3, 18.3-48.1)	35.4 (7.0, 24.0-54.2) *
Relative weight †, % (range)	-1.8 (-27.0-42.0)	6.5 (-12.0-48.0) *
Mean height, cm (SD, range)	136.4 (7.7, 119.2-154.9)	140.2 (6.6, 125.2-160.1) §
Relative height ‡, SD (range)	-0.5 (-3.0-2.2)	0.1 (-2.3-3.2) *

* p≤0.001 by t-test.
† Relative weight expressed as deviation of weight in % from the mean for height and sex (Pere 2000).
‡ Relative height expressed as deviation of height in SD units from the mean height for age and sex (Pere 2000).
§ p=0.003 by t-test.

Mean gestational age of the children born preterm was 28.5 weeks (SD 2.4, range 23.4-33.7), and mean birth weight was 1075 g. For the full-terms, mean birth weight was 3593 g (only two cases had weighed less than 2900 g) (Table 2). The neonatal data of the preterm group are described in Table 3. The gestational ages of children born at term were not recorded.

Table 3. Neonatal data on the preterm group (n=72).

Mean gestational age, weeks (SD, range)	28.5 (2.4, 23.4-33.7)
Antenatal steroid treatment, n (%)	2 (3)
Caesarean section, n (%)	40 (56)
Birth weight < -2SD *, n (%)	19 (26)
Human surfactant treatment †, n (%)	19 (26)
Respiratory distress syndrome, n (%)	49 (68)
Bronchopulmonary dysplasia, n (%)	33 (46)
Oxygen supplementation at 36 gestational weeks, n (%)	25 (35)
Parenteral dexamethasone treatment ‡, n (%)	14 (19)
Blood culture verified sepsis, n (%)	11 (15)
Mean length of hospital stay, months (SD, range)	3.0 (2.5, 1.0 – 14.2)

* Deviation of birth weight in SD units from the mean birth weight for gestational age and sex estimated by Finnish foetal growth curves (Pihkala et al. 1989).
† Treatment given in accordance with the ongoing study protocol (Merritt et al. 1991).
‡ Treatment given for weaning from ventilator.

Mean age at the time of examination was 10.1 years in both groups (Table 2). The relative height and weight were significantly lower in the preterm group than in the full-term group (Table 2). The groups did not differ significantly in month of birth, month of examination, number of siblings, parental smoking, furred pets at home, type of living area or socio-economic status of the family as judged from the educational level of the parents.

The proportion of atopic parents was similar in both groups, apart from maternal and paternal allergic rhinoconjunctivitis, which was more common in parents of children born full-term (Table 4). This result provided the basis for further analysis of the association between parental atopy and preterm birth (IV).

Respiratory infections during the first two years were more common in children born preterm than in those born full-term: 42% versus 15% (p=0.001). A corresponding difference was seen in the frequency of adenotomy and tympanostomy; 58% of the preterm group and 31% of the full-term group had undergone these operations (p=0.001).

Table 4. Proportions of parental atopic symptoms (%) in the two groups and differences between the groups.

Symptoms in one or both parents	Preterm group (n=72)	Full-term group (n=65)	p-value*	OR†	95% CI
Asthma, %	7	3	NS	2.35	0.44-12.55
Atopic eczema, %	29	31	NS	0.93	0.45-1.93
Allergic rhinoconjunctivitis, %	35	62	0.002	0.33	0.17-0.67
Family history of atopy ‡, %	51	66	0.080	0.54	0.27-1.08
Family history of definitive atopy §, %	15	22	NS	0.66	0.27-1.57
Symptoms in mother					
Asthma, %	4	3	NS	1.37	0.22-8.46
Atopic aczema, %	22	18	NS	1.26	0.55-2.92
Allergic rhinoconjunctivitis, %	24	38	0.06	0.49	0.24-1.05
Symptoms in father					
Asthma, %	3	0			
Atopic aczema, %	11	18	NS	0.55	0.21-1.45
Allergic rhinoconjunctivitis, %	14	31	0.017	0.36	0.15-0.85

* Chi-squared test.
† Logistic regression method.
‡ Asthma, allergic rhinoconjunctivitis and/or atopic eczema in one or both parents.
§ Asthma or symptoms in more than one organ in one or both parents.
NS = non-significant

The first introduction of foreign food antigen in preterm children was registered precisely from hospital case records. Early feeding data from the hospital records were available for 57 of the 72 preterm children. Cow's milk-based formula was introduced on day 36 (SD 27, range 9-149, median 26). Protein fortifier in breast milk or formula milk was given to 29 children at the mean age of 26 days (SD 14, range 10-74, median 22).

The above-described figures for the background factors apply to the entire groups of children participating in the studies. Minimal differences in distribution of these background factors between studies (I, II, III) are present because all children could not perform spirometry testing and a blood sample was either not given by all or was insufficient for food antibody measurements.

Groups of parents (IV)

Personal data on VLBW infants born in the three maternity hospitals in the Helsinki area (the Department of Obstetrics, Helsinki University Central Hospital; Helsinki City Maternity Hospital; and Jorvi Hospital) were collected from the registry of newborn infants, maintained at the Central Bureau of the Ministry of Health, Finland. From

November 1995 to the end of 1999, altogether 370 infants with a birth weight of less than 1500 g were born in these hospitals. Questionnaires concerning parental atopy were sent to the parents of each child.

Parents of 544 infants with a birth weight above 3000 g, born from July 1999 to the end of 1999 in the same hospitals, indentified in the same registry, were selected as controls. Questionnaires concerning parental atopy were also sent to the parents of these children.

The response rate to the questionnaires was 56% for both groups. Respondents comprised 213 mothers (58%) and 205 fathers (55%) of VLBW infants and 311 mothers (56%) and 305 fathers (56%) of full-term infants. Infants born from multiple pregnancies (6 full-term and 43 preterm) were excluded from the final analysis.

METHODS

Prenatal and neonatal data (I, II, III)

Prenatal and neonatal data on pregnancy, delivery, early medical problems, treatments and nutrition of children born preterm were collected from the hospital case records. The diagnosis of BPD was based on the criteria of Bancalari (1979). The diagnosis of CLD was not used at that time. The need for oxygen supplementation at 36 postconceptional weeks was, however, recorded referring to CLD (Shennan et al. 1988).

Questionnaire data (I, II, III, IV)

The parents of both groups of children were asked to fill out the questionnaire, including data on their own atopic symptoms, atopic symptoms and other diseases of their child and possible environmental risk factors for atopy. The questionnaire was checked and discussed in detail with the parent(s) at the outpatient clinic by the researcher (M.S.) (I, II, III).

When evaluating parental atopy and preterm birth, separate questionnaires were sent to both the mother and the father of each child, inquiring about parental symptoms suggestive of atopy (hay fever, asthma, atopic eczema/dermatitis) and whether any of their allergic diseases was diagnosed by a doctor (IV).

Diagnostic definitions (I, II, III, IV)

Atopic eczema was diagnosed when the child had a history of chronic or chronically relapsing itching dermatitis with typical morphology and distribution (Hanifin and Rajka 1980). Diagnosis of allergic rhinoconjunctivitis was based on a history of a runny and/or blocked nose and/or itchy, watery eyes with seasonal variation or with animal contacts and apart from infection episodes. Recurrent wheezing was confirmed when the child had a history of at least three episodes of wheezing. Breathing difficulties caused by acute laryngitis were excluded. Asthma was recorded when diagnosed by a doctor. The child was considered to have atopic asthma when, in addition to clinical asthma, at least one of the following objective findings of an atopic reaction was measured in the study: serum total IgE over 320 kU/l, at least one allergen-specific IgE level over 0.8 kU/l or at least one positive skin prick test (SPT) reaction.

The child was considered to be atopic if he/she had experienced at least one of the above-mentioned symptoms (symptoms in at least one organ). When the history of atopic symptoms was validated with at least one objective finding of an atopic reaction (as in the definition of atopic asthma), the child was classified as having obvious atopy.

A family history of atopy was defined as asthma, allergic rhinoconjunctivitis and/or atopic eczema in one or both parents (symptoms in at least one organ). A family history of definitive atopy was recorded when one or both parents had had asthma or symptoms in more than one organ.

In the study of parental atopy and preterm birth (IV), history of atopic symptoms (hay fever, asthma, or atopic dermatitis) was recorded separately for both parents. Atopy was classified as verified atopy when doctor-diagnosed.

Skin prick testing (I, II, III)

SPT was performed by two nurses at the hospital. The test consisted of a panel of eight standard Soluprick solutions® from ALK (Allergologiska Laboratorium, Copenhagen, Denmark); including extracts of birch, timothy, meadow fescue, mugwort pollen, cat and dog dander, house dust mite Dermatophagoides pteronyssinus, hen´s egg and latex Prick test standard solution® from Stallergenes SA (France). Histamine hydrochloride (10 mg/ml) served as a positive control and 50% glycerol solution as a negative control.

The reaction was regarded as positive when the wheal diameter was 3 mm or more and the negative control solution caused no reaction. All of the children participating in the study were tested.

Blood samples (I, II, III)

Serum total IgE concentration and allergen-specific IgE antibodies to hen´s egg, cat dander and birch pollen were measured by enzymatic UniCAP fluoroimmunoassay®, and serum eosinophil cationic protein (ECP) by UniCAP fluoroimmunoassay® (Pharmacia-Upjohn, Uppsala, Sweden). Serum total IgE over 320 kU/l, allergen-specific IgE antibodies over 0.8 kU/l and ECP concentration over 15 µg/l (II) were considered indicative of atopic sensitization. Blood eosinophil count was also measured.

Antibodies of IgA and IgG isotypes to cow's milk and its components β-lactoglobulin (BLG) and α-casein (ACAS), and ovalbumin were measured with an enzyme-linked immunosorbent assay (ELISA), with modifications (Saukkonen et al. 1994, Vaarala et al. 1995). Microtitre plates (Nunc Immunoplate®, Nunc A/S, Roskilde, Denmark) were coated with either diluted, defatted (1:500) adapted liquid milk formula (Tutteli®, Valio Ltd, Helsinki, Finland), with BLG (Sigma Pharmaceuticals, St. Louis, MO, USA) at a concentration of 1 µg/ml or with ACAS and ovalbumin (Sigma) at a concentration of 2 µg/ml in carbonate buffer, pH 9.6, overnight. Diluted sera were applied in triplicate to the antigen-coated plates and in duplicate to wells of the same microtitre plates coated with blocking solution (1% sheep serum); the plates were incubated overnight at room temperature. After washing, 75 µl of alkaline-phosphatase-conjugated monospecific swine anti-human IgG, IgA and IgM antisera (diluted 1:200) (Orion Diagnostica, Helsinki, Finland) were added, and the plates were incubated for 60 minutes at 37°C. After washing, 75 µl of p-nitrophenylphosphate substrate was added (2 mg/ml in diethanolamine buffer, pH 10.0, from I.T. Baker Chemical, Deventer, the Netherlands). The reaction was stopped after 30 minutes with 75 µl of 1 mol/l NaOH. The end-product was measured at 405 nm in a semi-automatic photometer (Titertek Multiscan®, Elflab Inc., Helsinki, Finland). The result was compared with that for 2-fold serial dilutions of a standard serum with a very high titre of the respective antibodies. The standard serum was given the value of 100 arbitrary units (AU). IgG gliadin antibodies were measured by ELISA (Kolho and Savilahti 1997).

IgG antibodies to tetanus and diphteria toxoids were measured with ELISA. Toxoids were a gift from Dr. Helena Käyhty (National Public Health Institute, Helsinki, Finland). Microtitre plates were coated with 100 µl of either toxoid at a concentration of 0.125 µg/ml (tetanus) or 0.25 µg/ml (diphtheria) in a 0.05 mol/l carbonate buffer, pH 9.6, and incubated overnight at 8°C. In the morning, these plates were washed with 0.05% Tween-PBS®, and blocking was done with 100 µl of 1% human serum albumin (Finnish Red Cross Blood Services, Helsinki, Finland) in PBS for one hour at room temperature. Sera were diluted in 0.2% human serum albumin in 0.05% Tween-PBS® to a concentration of 1:400 for tetanus-coated plates and 1:200 for diphteria-coated plates. Samples of 100 µl were applied to washed microtitre plates in duplicate and incubated for 2 hours at room temperature. After washing, 100 µl of alkaline phosphatase-conjugated anti-human rabbit IgG (Jackson Immuno Research) was added in a dilution 1:3000 in 0.05% Tween-PBS® with 0.2% human serum albumin. After incubation for 90 minutes at room temperature and washing, 100 µl of phosphatase substrate (p-nitrophenyl disodium phosphate, Sigma) diluted in 0.05 mol/l carbonate and 0.001 mol/l $MgCl_2 \bullet 6\ H_2O$ buffer, pH 9.6 (1 tablet/5 ml), was added. Microtitre plates were then incubated at room temperature for 30 minutes. The end-product was measured at 405 nm in a semi-automatic photometer (Titertek Multiscan®).

The standard was diphuman immunoglobulin, another gift from Dr. Helena Käyhty. It contained 65 IU/ml of tetanus antibodies and 201 IU/ml of diphtheria antibodies. The standard is annually calibrated at the National Health Institute with the World Health Organization's International Standard of Tetanus Human Immunoglobulin and the International Standard of Diphtheria Equine Antitoxin.

A blood sample was given by all 72 children of the preterm group (100%) and by 64 of the 65 children of the full-term group (98%). In analysis of food antibodies, the volume of the blood sample was sufficient for 62 in the preterm group (86 %) and for 61 in the term group (94%).

Spirometry testing (II)

Flow-volume spirometry testing with a spirometer (Vitalograph Compact Spirometer® Ltd., UK) was performed at rest and 10 minutes after an exercise challenge. At the testing, the children were sitting and wearing nose clips. Criteria for acceptance of the

recording were based on the guidelines of the American Thoracic Society (1994). During the exercise, which consisted of jumping on the trampoline for 6-8 minutes, the arterial pulse was monitored by a pulse meter (Polar Accurex®, Finland), and the arterial oxygen saturation by a pulse oxymeter (Nellcor N-20®, USA). The objective of the exercise was to produce breathlessness and a heart rate exceeding 170 beats per minutes for a 6-minute period. The following spirometry values were analysed: forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/ FVC ratio, forced expiratory flow after 50% of vital capacity has been exhaled (FEF50) and forced mid-expiratory flow of FVC (FEF25-75). The results were expressed and analysed as percentages of the predicted values adjusted for the age, sex and height of the child (Knudson et al. 1983).

Before the visit to the outpatient clinic, 16 of the 72 children in the preterm group (22%) and 5 of the 65 children in the full-term group (8%) had had a recent respiratory infection and thus were excluded from spirometry testing. Of the reminder, 6 in the preterm group (11%) and 6 in the full-term group (10%) were unable to perform spirometry acceptably according to the established criteria. Thus, spirometry testing was performed on a total of 50 children born preterm (69%) and 54 children born full-term (83%).

Statistical methods

Comparisons of categorical variables between the groups (environmental background factors, respiratory and atopic symptoms) were examined by Pearson´s chi-squared test or by Fisher´s exact test (I, II, III, IV). The following cut-point values were used to categorize some variables as dichotomous:

- 27 weeks' gestational age (II) or 30 weeks' gestational age (III)
- serum total IgE 320 kU/l (I, II, III)
- allergen-specific IgE antibodies 0.8 kU/l (I, II, III)
- serum ECP 15 ug/l (II)
- introduction of cow's milk-based formula before 50 days after birth (III)
- morbidity due to frequent respiratory infection (more than 6 episodes in 6 months) (I, II)
- more than one sibling (I, II)

- oxygen supplementation at 36 gestational weeks (I, II)

Between the groups of parents, comparisons of atopic symptoms were also performed with a trend test. For this test, three groups of mothers were formed: those with infants of birth weight above 3000 g, 1500-1000 g and below 1000 g (IV).

Spearman's rank correlation was used to analyse the correlation between the sum of three verified atopic diseases (allergic rhinitis, asthma, and atopic eczema) in the mother and birth weight of the child (IV).

Student´s t-test was used to compare continuous variables between the groups (age, height, and weight of the child, spirometry values, serum total IgE, blood eosinophil count, serum ECP, and serum antigen-specific IgG and IgA antibodies) (I, II, III). Right-skewed variables were log-transformed to normality (serum total IgE, blood eosinophils, serum ECP, serum antigen-specific IgG and IgA antibodies) (I, II, III). The Mann-Whitney U-test was used in non-normally distributed cases of continuous variables (I, II).

To analyse associations between food antibody levels and other data in either group of children, the sum of three ranks in the group was calculated for both the IgA and the IgG isotype of milk antibodies (whole cow's milk, BLG and ACAS) (III).

Logistic regression analyses were performed to evaluate the risk related to prematurity and atopic diseases (I, II) and the risk related to parental atopy and preterm birth (IV). The results have been given as odds ratios (ORs) and 95% confidence intervals (CIs). ORs with 95% CIs were also calculated for belonging to either the highest or the lowest quartile of IgA and IgG antibodies (III).

Multivariate stepwise logistic regression analyses with the forward selection method were performed to evaluate independent contributions of several background factors and to take into account the effect of potential confounding factors. The background factors for which distributions differed between the groups were also included in the model (infection morbidity, family history of atopy) (I, II).

For the study of parental atopy and preterm birth (IV), the sample size and power calculation were performed based on the results of the first part of the study, in which the prevalence of parental rhinoconjunctivitis was found to be significantly lower in the group of children born preterm. Hay fever among mothers of prematurely born children was 24% and among mothers of children born full-term 38%, resulting in a relative risk

of about 1.6. Thus, with a ratio of 1.5 between preterm and full-term babies, 349 preterm and 524 full-term babies were calculated to be needed to detect a risk ratio of 1.4 at a 5% level of significance and with 80% power.

The groups were unpaired. Test results were interpreted as statistically significant when the p-value was 0.05 or less. P-values ≤0.1 were indicated in the tables. P-values throughout the study were calculated as two-sided. Statistical analyses were done with SPSS Release 8.0 for Windows.

ETHICAL CONSIDERATIONS

The study was approved by the Ethics Committee of the Hospital for Children and Adolescents, University of Helsinki, and by the municipal authorities of the educational system of Helsinki and Kirkkonummi. Written consent was obtained from all families participating in the study.

RESULTS

ATOPY IN CHILDREN BORN PRETERM (I)

Atopic findings

The frequency of atopy was significantly lower in the preterm than in the full-term group; atopic symptoms were found in 31% of preterms versus 49% of full-terms (OR 0.45, 95% CI 0.23-0.91, p=0.026), and obvious atopy was observed in 15% of preterms and 31% of full-terms (OR 0.41, 95% CI 0.18-0.93, p=0.030). Frequencies of atopic eczema and allergic rhinoconjunctivitis separately analysed were also lower in the preterm than in the full-term group, but these differences did not reach statistical significance. Atopic asthma was equally common in both groups, whereas asthma without emphasis on atopy was more common in the preterm group (17% versus 8%, p=0.11) (Table 5).

Table 5. Proportions of cumulative incidences of respiratory and atopic symptoms (%) in the two groups and differences between the groups.

	Preterm group (n=72)	Full-term group (n=65)	p-value *	OR †	95% CI
Atopic eczema, %	22	35	0.088	0.52	0.25-1.11
Allergic rhinoconjunctivitis, %	19	34	0.056	0.47	0.22-1.03
Asthma, %	17	8	NS	2.40	0.80-7.23
Atopic asthma ‡, %	6	6	NS	0.89	0.21-3.74
Atopy §, %	31	49	0.026	0.45	0.23-0.91
Obvious atopy ‖, %	15	31	0.030	0.41	0.18-0.93
Recurrent wheezing ¶, %	43	17	0.001	3.71	1.67-8.25
Wheezing during the past year, %	12	12	NS	1.02	0.37-2.82

* Chi-squared test.
† Logistic regression method.
‡ Asthma with serum total IgE over 320 kU/l, at least one allergen-specific IgE level over 0.8 kU/l, or at least one positive skin prick test reaction.
§ Atopic eczema, allergic rhinoconjunctivitis, and/or atopic asthma (atopic symptoms in at least one organ).
‖ Atopic symptoms in at least one organ and serum total IgE over 320 kU/l, at least one allergen-specific IgE level over 0.8 kU/l, or at least one positive skin prick test reaction.
¶ Three or more episodes of wheezing.
NS = non-significant

In SPT, the children born full-term had two or three times more positive reactions to the tested allergens (Table 6). Comparing the frequencies of one, two and three positive SPT reactions to the nine allergens in the panel, significant differences in all categories were seen between the groups (p=0.007, p=0.045 and p=0.010, respectively). The same trend was observed when positivity for serum allergen-specific IgE antibodies was analysed (Table 6). The geometric mean of serum total IgE was significantly higher in the full-term group, 74.3 kU/l (95% CI 51.5-107.2) versus 40.7 kU/l (95% CI 28.9-57.4) (p=0.020). A corresponding trend was seen in serum ECP levels, 8.9 µg/l (95% CI 7.1-11.0) versus 6.8 µg/l (95% CI 5.7-8.1) (p=0.065).

Table 6. Proportions of children (%) in the groups with positive SPT reactions to nine standard test solutions, and children with high serum allergen-specific IgE levels (>0.8 kU/l), and the differences between the groups.

Positive SPT	Preterm group (n=72)	Full-term group (n=65)	p-value *
Birch, %	10	23	0.034
Timothy, %	8	20	0.069
Meadow fescue, %	11	26	0.023
Mugwort, %	3	9	NS
Cat, %	6	17	0.033
Dog, %	3	11	0.059
Dermatophagoides pteronyssinus, %	1	3	NS
Latex, %	1	0	NS
Egg, %	0	0	NS
At least 1 positive reaction, %	17	37	0.007
At least 2 positive reactions, %	14	28	0.045
At least 3 positive reactions, %	6	20	0.010
Serum-specific IgE measurement >0.8 kU/l	Preterm group (n=72)	Full-term group (n=64)	p-value *
Birch, %	11	20	NS
Cat, %	4	11	NS
Egg, %	0	3	NS
At least 1 of the above 3 measurements >0.8 kU/l, %	12	23	0.095

* Chi-squared test.
SPT = skin prick test.
NS = non-significant

The inverse association between prematurity and atopy remained significant when adjusted in multivariate analysis for parental atopy, smoking of parents, infection morbidity of the child, presence of furred pets at home and sex of the child. In the model, prematurity was associated with a decreased risk of obvious atopy (OR 0.42,

95% CI 0.18-0.98, p=0.046). Of the other variables in the model, only family history of atopy was significantly associated with atopy, increasing the risk (OR 2.74, 95% CI 1.06-7.08, p=0.037). The inverse association between prematurity and SPT-positivity was also seen in multivariate analyses (OR 0.34, 95% CI 0.15-0.76, p=0.008). Thus, the difference in the frequency of atopy between the groups could not be explained by possible confounding factors or by differences in any background factors.

Factors associated with atopy

The association of background factors (sex, respiratory infection morbidity during the first two years of life, parental atopy, educational level of parents, number of children in the family, furred pets at home, smoking of parents, type of home locality) with atopy was analysed for both groups. The only factor significantly associated with atopy in both groups was parental atopy (in the preterm group, OR 3.68, 95% CI 1.23-10.99, p=0.020; in the full-term group, OR 13.11, 95% CI 3.32-51.81, p=0.0002). Parental smoking during the first year of the child´s life was related to an increased risk of atopy in the full-term group (OR 3.28, 95% CI 1.11-9.70, p=0.032).

Parental smoking remained a significant risk factor of atopy in children born at term when analysed by the multivariate method (adjusted OR 14.1, 95% CI 1.5-132, p=0.02), as did parental atopy in both groups (preterm group, OR 3.62, 95% CI 1.2-10.9, p=0.02; full-term group, OR 46.0, 95% CI 4.8-441.4, p<0.001). The analyses were adjusted for all background variables, which were also analysed separately by univariate methods.

Perinatal and neonatal events and treatments during the intensive care period were investigated thoroughly in the preterm group, in the univariate analyses no single factor was found to be significantly associated with symptomatic or obvious atopy. The variables used in the analyses were gestational age; cause of preterm delivery; mode of delivery; duration of mechanical ventilation and oxygen therapy; RDS, BPD, blood culture-verified sepsis; feeding with cow´s milk-based formula or protein fortifier before 2 weeks of age; and length of hospital stay.

ATOPY IN RELATION TO RESPIRATORY SEQUELAE IN CHILDREN BORN PRETERM (II)

Wheezing, asthma and atopy

Cumulative incidence of wheezing was significantly higher in the group of children born preterm than in the group of children born full-term, 43% versus 17% (OR 3.71, 95% CI 1.67-8.25, p=0.001), but the frequency of wheezing during the past year was equally common in both groups, 12% (Table 5). Thus, in the preterm group, wheezing significantly improved with age; only 26% (8/31) of these wheezers still wheezed (plus one new case), whereas in the full-term group, 73% (8/11) of wheezers still wheezed (p=0.011).

In the preterm group, cumulative incidence of wheezing (but not asthma) was significantly associated with low gestational age, RDS, BPD and oxygen supplementation at the gestational age of 36 weeks (p=0.039, p<0.001, p<0.001 and p=0.002, respectively), but not with intrauterine growth retardation, parenteral dexamethasone or surfactant treatment.

The association of atopy with cumulative incidence of wheezing was significantly different between the groups according to several indicators of atopy. More than 60% of wheezers in the full-term group had had some atopic findings; the corresponding proportion in the preterm group was only 20-30%. Wheezers in the full-term group also had a positive family history of atopy significantly more often than wheezers in the preterm group (Table 7).

Table 7. Proportions of children (%) in the non-wheezer and wheezer subgroups. Difference in the frequency of atopic findings between non-wheezers and wheezers within the groups, and between wheezers of the two main groups.

	Preterm group (n=72)			Full-term group (n=65)			
	Non-wheezers (n=41)	Wheezers (n=31)	p-value *	Non-wheezers (n=54)	Wheezers (n=11)	p-value *	Difference between wheezers of the two groups p-value
Asthma, %	5	32	0.002	0	46	<0.001	NS
Allergic rhinoconjunctivitis, %	12	29	NS	24	82	0.001	0.004
Obvious atopy, %	10	23	NS	24	64	0.026	0.024
Prick test positive, %	12 †	23	NS	32 †	64	NS	0.024
Specific S-IgE(s) >0.8 kU/l, %	7	19	NS	17	60	0.008	0.040
S-ECP >15 µg/l, %	17	13	NS	18	50	0.046	0.025
Parental asthma, %	7	6	NS	2	9	NS	NS
Parental atopy, %	51	52	NS	61	91	NS	0.030

* Difference between non-wheezers and wheezers within the main group.
† Difference between non-wheezers of the two main groups also significant, p=0.027.
NS = non-significant

Within the preterm group, no significant difference existed in the frequencies of atopic findings listed in Table 7 between wheezers and non-wheezers, whereas in the full-term group wheezing was significantly associated with allergic rhinoconjunctivitis, obvious atopy, high serum-specific IgE value and high serum ECP value.

In the preterm group, wheezing was related to respiratory infections more often than in the full-term group, 94% versus 64% (OR 8.28, 95% CI 1.25-54.7, p=0.032), but less frequently to pollen season (19% vs. 54%, OR 0.20, 95% CI 0.05-0.88, p=0.049) or to animal contact (3% vs. 36%, OR 0.06, 95% CI 0.01-0.61, p=0.013). Exercise was reported to have caused wheezing equally often in both groups, in 42% of the wheezers in the preterm group and in 46% of the wheezers in the full-term group. No significant association was found in either group between wheezing and exposure to tobacco smoke or to furred pets in the home.

Of those who had wheezed during the past year, in both groups atopic individuals comprised the majority. Within the full-term group, 75% of those who still wheezed in the past year had obvious atopy in comparison with 33% of those who no longer wheezed (p=0.49). In the preterm group, the corresponding figures were 62% and 9% (p=0.006). None of the neonatal factors was significantly associated with persistence of wheezing at 10 years of age.

Lung function and atopy

In spirometry testing, the preterm children at rest exhaled significantly lower values in all of the measured variables (Table 8). During the exercise challenge two children, both from the preterm group, developed manifest wheezing and one of these children also had major changes in lung function values measured after the exercise. However, the overall changes in FEV1, FEF50 and FEF25-75 postexercise did not differ significantly between the groups (Table 8). Nor did the mean arterial pulse or the arterial oxygen saturation differ between the groups during exercise.

In the preterm group, history of wheezing was significantly associated with lower values of FEV1 and FEF50 ($p \leq 0.001$). Low gestational age, RDS, BPD and oxygen supplementation at the gestational age of 36 weeks were significantly related to lower values of FEV1, FEF50 and FEF25-75 ($p \leq 0.01$). Atopic children in the preterm group showed a trend towards lower lung function values than non-atopic children, but the

difference was not significant. In the full-term group, neither respiratory symptoms nor atopy were associated with spirometry values. Parental atopy, asthma and smoking were not significantly related to lung function in either group.

Table 8. Spirometry results and differences between groups.

	Preterm group (n=50)	Full-term group (n=54)
FVC, % of predicted ±SD	96 ±12.6	102 ±9.6 *
FEV1, % of predicted ±SD	92 ±13.1	104 ±8.0 †
FEV1/FVC, % ±SD	84 ±7.8	88 ±5.4 †
FEF50, % of predicted ±SD	82 ±21.6	106 ±20.7 †
FEF25-75, % of predicted ±SD	87 ±24.0	114 ±21.2 †
FEV1 change, % after exercise ±SD	-1.5 ±10.2	-0.8 ±3.1
FEF50 change, % after exercise ±SD	-4.3 ±15.1	-1.6 ±10.1
FEF25-75 change, % after exercise ±SD	-1.3 ±16.3	-3.4 ±11.5

* p=0.016 by t-test.
† p≤0.001 by t-test.
FVC = forced vital capacity, FEV1 = forced expiratory volume in one second, FEF50 = forced expiratory flow after 50% of vital capacity has been exhaled, FEF25-75 = forced mid-expiratory flow of FVC.

ANTIBODY LEVELS TO EARLY-INTRODUCED FOOD ANTIGENS IN CHILDREN BORN PRETERM (III)

IgG and IgA antibody levels

Children in the preterm group were found to have significantly lower levels of IgG isotype antibodies to whole cow's milk and ACAS, with geometric mean levels being about one-fourth of those in the term group (Table 9). Measurable IgG levels to BLG were significantly less frequent in the preterm group than in the term group. IgA antibodies to whole cow's milk and ACAS were also lower in the preterm group, whereas the proportions of children with measurable levels of IgA antibodies to BLG showed no difference between the groups. IgG antigliadin levels were lower in the preterm group, and the same tendency was observed for IgG ovalbumin antibodies (Table 9).

The preterm and term groups had similar geometric mean levels of IgG antibodies to tetanus (55.7 IU/ml $x10^{-3}$ with 95% CI 54.1-57.4 IU/ml$x10^{-3}$ and 45.3 IU/ml$x10^{-3}$

with 95% CI 33.4-61.5 IU/mlx10^{-3}, respectively) and diphtheria toxoids (20.0 IU/mlx10^{-3} with 95% CI 15.4-26.1 IU/mlx10^{-3} and 16.7 IU/mlx10^{-3} with 95% CI 12.9-21.7 IU/mlx10^{-3}, respectively).

Factors associated with antibody levels

Within the preterm group, gestational age was associated with cow's milk antibody levels: those born before week 30 had significantly lower levels of IgA cow's milk antibodies, and the proportion with positive IgG isotype antibodies to BLG among these patients was lower than among those born between 30 and 34 gestational weeks (Table 9).

Those children in the preterm group who had consumed cow's milk formula before age 50 days had significantly lower levels of IgA antibodies to cow's milk and ACAS than did those started on formula after day 50 (Table 9).

The children in the preterm group with obvious atopy had significantly lower levels of cow's milk IgG antibodies (0.7 AU with 95% CI 0.2-2.8 vs. 3.6 AU with 95% CI 2.5-5.3, p=0.04) and ACAS IgG antibodies (0.2 AU with 95% CI 0.1-0.4 vs. 0.8 AU with 95% CI 0.5-1.3, p=0.02) than did those without atopic symptoms. They did, however, show higher levels of ovalbumin IgG antibodies than did children without atopic symptoms (162 AU with 95% CI 114-230 vs. 36 AU with 95% CI 22-61, p<0.001). In the term group, no difference was apparent between children with obvious atopy and those with no symptoms of atopy.

Logistic regression analyses showed a significant OR for preterm children with atopic symptoms being in the lowest quartile for IgG cow's milk antibodies (OR 11.9, 95% CI 1.6-88, p=0.002). Significant ORs for being in the lowest quartile of IgA cow's milk antibodies were related to early introduction of cow's milk formula (before day 50; OR 13, 95% CI 1.2-144, p=0.04) and gestational age of less than 30 weeks (OR 11, 95% CI 1.1-120, p=0.04).

Table 9. Geometric mean levels with 95% confidence intervals (CI) of IgA and IgG antibodies to cow's milk and α-casein, of IgG antibodies to gliadin and ovalbumin in arbitrary units (AU) and the proportion of positive IgA and IgG titres to β-lactoglobulin (BLG) in 10-year-old children who were born preterm or full-term.

	Full-term group (n=61)	Preterm group (n=62)	p-value	Preterm group					
				Born before gestational week 30 (n=40)	Born after gestational week 30 (n=22)	p-value	Milk-based formula before age 50 days (n=36)	Milk-based formula after age 50 days (n=24)	p-value
Cow's milk IgA (AU), Mean (95% CI)	1.0 (0.7-1.5)	0.3 (0.2-0.5)	< 0.001*	0.2 (0.1-0.4)	0.6 (0.3-1.5)	0.03*	0.2 (0.1-0.4)	0.5 (0.3-1.0)	0.03*
Cow´s milk IgG (AU), Mean (95% CI)	9.2 (7.0-12.0)	2.4 (1.6-3.6)	< 0.001*	2.0 (1.3-3.2)	3.3 (1.5-7.3)	0.08*	1.7 (1.0-3.1)	3.2 (1.8-3.2)	NS*
α-casein IgA (AU), Mean (95% CI)	2.0 (1.3-3.1)	0.5 (0.3-0.8)	< 0.001*	0.4 (0.2-0.6)	0.9 (0.4-2.1)	0.09*	0.3 (0.2-0.6)	0.8 (0.4-0.8)	0.04*
α-casein IgG (AU), Mean (95% CI)	2.4 (1.8-3.3)	0.48 (0.3-0.8)	< 0.001*	0.4 (0.2-0.7)	0.7 (0.3-1.9)	NS*	0.3 (0.2-0.6)	0.8 (0.4-1.6)	NS*
BLG IgA, proportion with positive titre (%)	57	45	NS†	45	48	NS†	40	54	NS†
BLG IgG, proportion with positive titre (%)	90	59	<0.001†	50	76	0.05†	54	67	NS†
Gliadin IgG (AU), Mean (95% CI)	3.2 (2.3-4.6)	1.7 (1.1-1.7)	0.03*	1.4 (0.8-2.5)	2.4 (1.1-5.3)	NS*			
Ovalbumin IgG (AU), Mean (95% CI)	74 (53-103)	48 (32-71)	0.1*	46 (27-80)	49 (28-85)	NS*			

* By t-test.
† By Chi-squared test.
NS = non-significant.

MATERNAL ATOPY AND PRETERM BIRTH (IV)

Mothers of VLBW infants reported having less atopic symptoms and less doctor-diagnosed atopic diseases than mothers of infants born at term (Table 10). Among mothers of ELBW infants, a significant correlation was present between the infant's birth weight and the number of verified atopic diseases in the mother (Spearman's correlation coefficient = 0.29, p=0.009). The OR for mothers of ELBW infants showed a significantly decreased risk of having doctor-diagnosed allergic rhinitis (OR 0.49, 95% CI 0.26-0.89) (Table 11).

Table 10. Allergic symptoms and doctor-diagnosed allergies in mothers and fathers of preterm infants of very low birth weight (<1500 g, VLBW) and of full-term infants. Infants of birth weight (BW) below 1000 g (extremely low birth weight, ELBW) and between 1000 and 1500 g are analysed separately.

Type of allergy	ELBW infants <1000 g	Infants with BW 1000-1500 g	All VLBW infants <1500 g	Full-term infants >3000 g
Mothers	(n=76)	(n=94)	(n=170)	(n=306)
History of allergic rhinitis, %	45	51	48	55
Diagnosed allergic rhinitis, %	20	32	27	34
History of asthma, %	15	17	16	19
Diagnosed asthma, %	12	14	13	15
History of atopic dermatitis, %	29	41	36	39
Diagnosed atopic dermatitis, %	24	33	29	33
Fathers	(n=76)	(n=89)	(n=165)	(n=301)
History of allergic rhinitis, %	42	45	44	46
Diagnosed allergic rhinitis, %	26	21	24	27
History of asthma, %	17	16	16	18
Diagnosed asthma, %	16	10	13	14
History of atopic dermatitis, %	30	28	29	31
Diagnosed atopic dermatitis, %	25	22	23	23

When analysed with a trend test, the probability of doctor-diagnosed allergic rhinitis in the mother was significantly higher when the infant's birth weight was greater (p=0.03). A similar trend was also observed for self-reported symptoms of allergic rhinitis (p=0.1). No such differences were evident for atopic symptoms and doctor-diagnosed atopic diseases in the fathers of the same infants (Table 11).

Table 11. Unadjusted odds ratios (OR) and 95% confidence intervals (CI) showing the risk of parental atopy on preterm birth, and p-value of the test for trend.

		Mothers			Fathers		
		%	**OR**	**95% CI**	**%**	**OR**	**95% CI**
History of allergic rhinitis	BW <1000 g	45	0.66	0.40-1.09	42	0.85	0.51-1.42
	BW 1000-1500 g	51	0.85	0.53-1.35	45	0.96	0.60-1.54
	Full-term	55	1		46	1	
	Test for trend (p-value)		0.1			0.55	
Doctor-diagnosed allergic rhinitis	BW <1000 g	20	0.49	0.26-0.89	26	0.95	0.54-1.69
	BW 1000-1500 g	32	0.92	0.56-1.51	21	0.73	0.41-1.28
	Full-term	34	1		27	1	
	Test for trend (p-value)		0.03			0.62	
History of asthma	BW <1000 g	15	0.74	0.37-1.49	17	0.97	0.50-1.88
	BW 1000-1500 g	17	0.91		16	0.87	0.46-1.66
	Full-term	19	1		18	1	
	Test for trend (p-value)		0.40			0.82	
Doctor-diagnosed asthma	BW <1000 g	12	0.78	0.36-1.67	16		0.59-2.39
	BW 1000-1500 g	14	0.94	0.48-1.84	10		0.33-1.53
	Full-term	15	1		14	1	
	Test for trend (p-value)		0.54			0.87	
History of atopic dermatitis	BW <1000 g	29	0.63	0.37-1.09	30		0.56-1.66
	BW 1000-1500 g	41	1.07	0.67-1.71	28		0.51-1.46
	Full-term	39	1		31	1	
	Test for trend (p-value)		0.17			0.76	
Doctor-diagnosed atopic dermatitis	BW <1000 g	24	0.64	0.36-1.15	25	1.11	0.62-1.99
	BW 1000-1500 g	33	1.00	0.61-1.65	22	0.93	0.52-1.66
	Full-term	33	1		23	1	
	Test for trend (p-value)		0.19			0.81	

BW = birth weight

DISCUSSION

ATOPIC IMMUNE RESPONSES IN CHILDREN BORN PRETERM

Atopy and prematurity

The main outcome of the study was that children born preterm bore a decreased risk of atopic sensitization; cumulative incidence of symptomatic atopy was significantly lower in the group of prematurely born children than in the group of children born at term. It is noteworthy that the frequency of each atopic symptom separately analysed also reflected the same tendency of lower incidence in the preterm group, although these differences did not reach the pre-defined level of statistical significance. Parallel results in all IgE-related measurements in the study (number of positive SPT reactions, serum total IgE and ECP levels) strengthened the validity of the outcome.

Frequencies of atopic diseases recorded here in the full-term group are consistent with the recent results of population-based epidemiological studies, in which the lifetime prevalence of allergic disorders and their regional differences in Finnish paediatric populations were evaluated (Pekkanen et al. 1997, Remes et al. 1998). According to these studies, of 11 607 children aged 13-14 years, 44% had had at least one atopic disorder; in the Helsinki area, the corresponding figure was 48%. The lifetime prevalence of self-reported asthma was 4-8%, allergic rhinitis 44-55% and itching dermatitis 24-28% (the ranges are related to the different geographical regions where the study was carried out). In our full-term group, the corresponding figures were 49%, 8%, 34% and 35%, the frequency of allergic rhinitis being slightly lower and the frequency of atopic eczema slightly higher than in the population-based study.

Previous studies evaluating the relation between prematurity and atopy have yielded inconsistent results. A group of earlier studies, listed in Table 1a, describe findings in complete contradiction to ours. These studies have reported a positive association between prematurity or low birth weight and different variables of atopy, leading to the assumption that prematurely born children are at risk for contracting

atopic diseases. These results can be interpreted as suggesting that immaturity of immune responses and increased permeability of the gut during early antigen exposure leads to sensitization rather than development of tolerance, as shown in rodent studies of tolerance (Strobel and Ferguson 1984, Miller et al. 1994).

The general view of the consequences of early life exposure has during recent years evolved, and antigen exposure during the neonatal period is currently thought to be a tolerogenic rather than an immunogenic event. Consequently, preterm children who are immaturely exposed to the extra-uterine environment, can be hypothesized to have a decreased risk of atopic diseases. Our findings support this hypothesis and are in agreement with several other recent studies presented in Table 1c. The inverse association between prematurity and atopy was first noted by David and Ewing (1988) as a lower number of children born preterm among patients suffering from severe atopic eczema. Ten years later in Sweden, Bråbäck and Hedberg (1998) performed an epidemiological study by linking the perinatal and atopic data from two registers of 149 398 male conscripts. They found that both gestational age of less than 33 weeks (OR 0.75, 95% CI 0.61-0.91, p=0.0001) and VLBW (OR 0.69, 95% CI 0.49-0.97, p<0.0001) were significantly related to a reduced risk of allergic rhinitis. The study included large numbers of subjects with VLBW (<1500 g, n=362) and subjects born very prematurely (<33 gestational weeks, n=1269), suggesting a high validity for the outcome. They speculated that there might be less pronounced foetoplacental Th2 cell skewing in pregnancies that terminate prematurely, and thus, babies born of these pregnancies could have a more Th1 cell-deviated immune balance.

A recent study from Sweden (Mai et al. 2003) had a research protocol closely resembling that of the present study, including similar aims, inclusion criteria, size of the cohorts, year of birth and age of the children and evaluation methods. The authors also measured the secretion of IL-4, IL-5 and IFN-γ by PBMCs. Despite the congruence of the research protocols, results in that study were different from ours: they found no significant association between VLBW and markers of atopy. Only asthma was reported to be more frequent in VLBW children. Martino et al. (1989) also performed a similar research in Italy, but in that study the children were younger and their state of immaturity was less pronounced. Neither this study nor the epidemiological studies described in Table 1b describe any significant association between frequencies of atopic findings and gestational age or birth weight. These results lead to the assumption of an

equal functional capacity to develop tolerance or sensitization irrespective of immunological maturity stage during the neonatal period.

Direct comparisons of our results and those of previous studies evaluating the association between prematurity or low birth weight and atopy are hampered by the lack of uniformity in study protocols. Differences in subjects, methods and outcome variables are often considerable and must be taken into account. One special problem complicating comparison relates to the variables of low birth weight and low gestational age. While these variables often widely overlap, they may also reflect different developmental aspects and have different consequences. Low birth weight is related to prematurity but may also reflect intrauterine growth retardation. Infants of low gestational age, on the other hand, may have a birth weight either appropriate or low (only rarely high) for gestational age.

Atopy and long-term respiratory sequelae

We demonstrated a significant difference in the background factors of wheezing and asthma between children born preterm and those born at term. Within the full-term group, over half of the wheezers had had some atopic manifestations and over 90% had a positive family history of atopy (Table 7), whereas in the preterm group the cumulative incidence of wheezing had no significant association with any atopic finding or with parental atopy. The difference between the groups was also reflected in the factors causing wheezing: wheezers in the full-term group had symptoms significantly more frequently in relation to typical allergen contacts, such as exposure to pollen and animals, whereas symptoms in wheezers in the preterm group were more often related to respiratory infections.

The role of atopy in wheezing of children born preterm compared with children born full-term has not been previously evaluated. In some studies focusing on respiratory morbidity in preterm children, the frequency of atopy was reported, but was not compared with the corresponding frequency in controls (Chan et al. 1989a, Pelkonen et al. 1997). These studies found no significant association between respiratory symptoms and SPT reactions in the groups of preterm children. In the study of late pulmonary sequelae of BPD, Northway et al. (1990) analysed the frequency of

atopy between groups of preterm children and controls but did not link the result to respiratory findings.

The significant association found here between atopy, asthma and wheezing in the full-term group is in agreement with the results of several previous studies in which unselected populations of older children and young adults were evaluated (Burrows et al. 1989, Sears et al. 1991, Peat et al. 1996, Strachan et al. 1996). In infants, however, as Martinez et al. (1995) have demonstrated, a considerable proportion of wheezing is non-atopic and transient, is related to a diminished airway function at birth and becomes symptomatic especially during viral infections. Wheezing in prematurely born children can be considered to be more parallel to this type of wheezing. The following findings support this argument:

1. Children of the preterm group significantly more frequently had recurrent wheezing especially in relation to respiratory infections.

2. The cumulative incidence of wheezing in the preterm group was not associated with atopy.

3. Wheezing in the prematurely born children was transient. These children tended to recover, as also demonstrated in a few earlier studies (Kitchen et al. 1992, Blaney et al. 2001). The prevalence of current wheezing was only 12% in the group of preterm children, being the same in the full-term group.

An increased risk of long-term pulmonary sequelae in children born preterm has also been shown in several previous studies (Kitchen et al. 1992, Rona et al. 1993, Elder et al. 1996, McLeod et al. 1996, Gross et al. 1998). Prematurity and low birth weight have therefore been widely considered significant risk factors for childhood wheezing and asthma (Seidman et al. 1991, Arshad et al. 1993, Frischer et al. 1993, von Mutius et al. 1993, Shaheen et al. 1999, Katz et al. 2003, Mai et al. 2003), although the opposite conclusion has also been reached (Sears et al. 1996, Fergusson et al. 1997, Gregory et al. 1999, Leadbitter et al. 1999, Räsänen et al. 2000, Sin et al. 2004).

In the preterm group, several intrinsically correlated neonatal variables relating to severe immaturity (low gestational age, RDS, BPD, oxygen supplementation at the gestational age of 36 weeks) were significantly associated with the cumulative incidence of wheezing but not with current wheezing. The only prognostic factor for current wheezing in children born preterm was atopic predisposition, similar to in children born full-term. This finding suggests that those in the preterm group who

wheezed at the age of 10 years belong to a different subgroup of wheezing than the majority of the preterm wheezers. The retrospective approach of this study, unfortunately, did not allow for analysis of age at onset of wheezing.

Prematurely born children exhaled significantly lower spirometry values in all measured parameters. The means of these values in both groups were within normal limits, but as SD values and the significant difference between the groups reflect, children of the preterm group more frequently exhaled results classified as abnormal (e.g. with regard to FEV1 value, 16 children of the preterm group exhaled a result of less than 80% of the expected, whereas in the full-term group only 1 child did).

Impaired lung function in children born preterm has also been reported in many previous studies (Bertrand et al. 1985, Chan et al. 1989b, Northway et al. 1990, von Mutius 1993, Rona et al. 1993, McLeod et al. 1996, Pelkonen et al. 1997, Kennedy et al. 2000). These results indicate obstructed airways and possibly reduced total lung capacity or air-trapping. In the preterm group, wheezing, asthma and several neonatal factors that were correlated with the frequency of wheezing were also associated with impaired lung function, as also shown in earlier reports (Chan et al. 1989b, von Mutius et al. 1993, Parat et al. 1995, McLeod et al. 1996, Pelkonen et al. 1997, Kennedy et al. 2000). Neither atopic predisposition of a child nor parental atopy, asthma or smoking was significantly associated with lung function in either group. However, the low number of subjects completing spirometry testing may have prevented the detection of weak associations.

In the exercise challenge test, no significant differences were present between the groups. This corresponds with the finding of similar histories of exercise-induced wheezing in the groups, but disagrees with the results of a few previous studies which have demonstrated exercise-induced bronchial hyperreactivity in children born preterm (McLeod et al. 1996, Bader et al. 1987, Gross et al. 1998). The form of the exercise challenge in our study may have limited detection of changes; the exercise was performed by jumping on a trampoline, and spirometry blowing was performed after the exercise only once, at 10 minutes post-exercise.

The data of the present study demonstrate no significant association between prematurity and classical atopic asthma. The result is not surprising when considered from the clinical viewpoint but does indicate the diversity of the background of wheezing conditions and brings into question the validity of direct comparisons of all

wheezing conditions without subgrouping. More precise nomenclature for respiratory problems and asthma is called for.

Food antigen responses

Immaturity at birth was found to have a significant effect on food antigen-induced immune responses, the effect still being measurable after 10 years. Children born preterm had markedly lower levels of IgA and IgG antibodies to cow's milk and to its protein fractions. IgG gliadin antibodies were also significantly lower, but antibody levels to ovalbumin did not differ between the groups. The result is in agreement with an earlier study reporting decreased production of specific IgG and IgM antibodies to cow's milk in premature infants (Helms and Rieger 1987). The children in that study were, however, much younger than the children in the preterm group here. The immaturity of the immune and intestinal systems of VLBW infants appears to result in suppression of immune response to oral antigens given during early life, thereby making the B cells of these children more tolerant to food antigens. The finding that these same preterm children had less atopy may be another facet of their tolerance development, possibly reflecting an inherent susceptibility of immature T cells to induce tolerance, as speculated by Takahashi et al. (1995). Results of rodent studies, however, diverge from this hypothesis (Strobel and Ferguson 1984, Miller et al. 1994). It is thus probable that maturational status is not a main determining factor in development of oral tolerance. In different circumstances, neonatal naive T cells irrespective of immaturity can be either immunized, tolerized or switched to Th1 or Th2 responses depending on the dose of the antigen, the condition under which it is introduced, the type of adjuvant and the type of APC (Ridge et al. 1996).

The difference in antibody levels between preterm and full-term children was dependent on the age at introduction of the food antigen. The difference was strongest with early-introduced cow's milk-based formula, less strong with later-introduced cereal and non-significant with egg extract, which is generally introduced much later to the diet. The effect of age at introduction was evident even within the preterm group; the earlier the cow's milk-based formula was introduced, the lower the antibody levels. In previous studies of full-term children (Tainio et el. 1988, Jenmalm and Björksten 1998), the effect has been shown to be the opposite; when cow's milk-based formula was

introduced early, titres were higher. The result of the present study may reflect the role of maturation of the immune system in antibody production capacity and a rapid change in intestinal permeability in preterm infants.

The preterm and term children showed similar levels of IgG antibodies to tetanus and diphteria toxoids. This indicates that children born preterm did not have a generalized B cell defect. In several earlier studies, prematurely born children have been shown to develop adequate vaccine-induced antibody levels for protection against a particular infection, but the antibody levels have, however, remained significantly lower than in term infants (Faldella et al. 1998, Schloesser et al. 1999, Kirmani et al. 2002), possibly reflecting lower general antibody production capacity.

The pathogenetic role of food antibodies in a number of food-related diseases is poorly understood. Aberrant immune responses to food antigens may be important in the development of some autoimmune diseases, such as type I diabetes (Vaarala et al. 1999). For food allergy and atopy, IgG antibodies are widely reported to have no predictive value (Fälth-Magnusson et al. 1988, Tainio et al. 1988, Burks et al. 1990, Host et al. 1992, Barnes 1995, Keller et al. 1996). Some studies have, however, described an association between high IgG and IgE responses and development of atopic diseases (Jenmalm and Björksten 1998, Eysink et al. 1999, Oldaeus et al. 1999). In the preterm group here, the IgG isotype food antibodies were associated with atopy. In atopic preterm children, IgG antibodies to milk were lower and those to ovalbumin higher than in non-atopic children. This result may be related to the rapid postnatal decrease in intestinal absorption demonstrated previously (Kuitunen et al. 1994).

POSSIBLE AETIOLOGICAL FACTORS EXPLAINING DIFFERENCES IN IMMUNE RESPONSE OF PREMATURELY BORN CHILDREN

Prenatal environment

The underlying conditions causing preterm delivery may influence immunological maturation of the foetus. Pre-eclampsia induces several effects on the foetus, but of special interest here is cortisol production because cortisol has a marked and complex

regulatory influence on the immune system. In pre-eclampsia, the foetus is stressed and the mean foetal plasma cortisol concentration is significantly higher than in normal pregnancies (Goland et al. 1995). In addition, glucocorticoids are also used both antenatally in treatment of pre-eclampsia for accelerating foetal pulmonary maturation and postnatally in treatment of BPD/CLD; in the preterm group here, 3% had been treated antenatally by steroids and 19% had been dosed with parenteral dexamethasone for weaning from the ventilator (Table 3). Subjects with atopic dermatitis and/or asthma have, by contrast, been shown to have significantly attenuated cortisol responses to stress (Buske-Kirschbaum et al. 2002, 2003). Together these data suggest that a higher concentration of plasma cortisol during the perinatal period may have a role in the development of Th cell balance and in subsequent atopic sensitization of preterm children.

A large proportion of preterm deliveries are caused by a foetomaternal infection, chorioamnionitis, which may in part explain the lower frequencies of atopy in these children. The above-described theory related to cortisol may also be applied to perinatal infections, as infections cause significant stress to the foetus. However, a more up-to-date explanation is a microbial-burden aetiology, which is based on the proven inverse association between infection morbidity and atopy (Shaheen et al. 1996, Matricardi et al. 1997, Holt et al. 1999, von Mutius et al. 2000), first presented by Strachan (1989) as a hygiene hypothesis.

In this study, the causes of preterm birth included pre-eclampsia (22%), premature rupture of membranes (26%), chorioamnionitis (24%), multiple gestation (12%) and placental ablation (14%); in 25% of cases, the cause was something else or unknown. These conditions could exist concurrently. The aetiology of preterm delivery was, however, not significantly associated with atopy. This may be related to the low number of cases.

Mode of delivery

Caesarean section has been found to increase the risk of asthma of offspring in adulthood (Xu et al. 2001), and it has even been related to an increased risk of food allergy (Eggesbo et al. 2003). However, Lucas et al. (1990) reported that preterm infants born by Caesarean section had less wheezing and asthma. Caesarean delivery may

influence the infant's immune system in several ways (Grönlund et al. 1999a), one of which is by changing the gut microflora; gut flora of infants born by Caesarean section has been shown to be disturbed for up to 6 months after birth (Grönlund et al. 1999b). This may have an effect on development of atopy because gut microflora is known to be associated with atopic sensitization (Sudo et al. 1997, Björksten et al. 2001, Kalliomäki et al. 2001a).

Of the preterm children in this study, 56% were born by Caesarean section (Table 3). If the above-mentioned positive associations between Caesarean section and asthma and food allergy are valid, this would increase the risk of atopy in the preterm group. It is, however, probable that the effect of Caesarean section on atopy is weaker than the effects of other environmental factors that burden preterm children. In the preterm group here, the mode of delivery was not associated with atopy.

Postnatal environment

The mean length of the hospital stay of preterm children was 3.0 months (Table 3). Thus, preterm infants throughout their neonatal period are exposed to a different environment (regimen and timetable of feeding, aero-allergen exposure, hospital microbial colonization) than their term-born healthy counterparts. This can be speculated to have a role in the conversion of Th cell balance.

Of the preterm children, 15% had had blood-culture verified sepsis (Table 3), with many others likely to have experienced septicemia without laboratory confirmation. The frequency can be assumed to be significantly higher than among full-term infants. As discussed in the context of chorioamnionitis, the early infections may play a part in the development of sensitization. Neonatal bacterial infections of preterm infants can be hypothesized to affect Th cell balance by adjusting it more towards a Th1 cell-type profile, and thus protecting against allergen-specific Th2 cell reactions later in life.

Use of antibiotics, on the other hand, has been shown to disturb gut flora, which has been speculated to prevent postnatal Th1 cell maturation (Oyama et al. 2001, McKeever et al. 2002). With regard to preterm infants, who are often repeatedly dosed with antibiotics, this could antagonize the atopy-preventing effects of infection.

Maternal atopy and preterm birth

During pregnancy a Th2-deviated immune balance dominates in the maternal and foetoplacental immune systems, and this is considered to be fundamental for the normal progression of pregnancy (Wegmann et al. 1993). Th2-deviated balance also dominates in atopic individuals. The question therefore arises of a possible accentuating effect of maternal atopic status on pregnancy-related Th2 balance. Results of study IV on maternal atopy and preterm birth support this hypothesis; mothers of infants born at term had significantly more allergic rhinitis than mothers of infants born severely preterm. Observations of study I, which compared the family history of atopy between the preterm and the full-term children, are also congruent with this theory. Consistent findings in these two independent cohorts strengthen the conclusion.

The finding is in agreement with the result of von Mutius et al. (1993), who observed that a family history of hay fever or eczema was more frequent among term than preterm boys (39% vs. 27%, $p<0.05$). However, divergent results have also been presented. Chan et al. (1989a) and Northway et al. (1990) found no difference in family history of atopy between low birth weight and reference groups.

Nilsson et al. (1997) approached this question from an interesting viewpoint. They hypothesized that Th2-dominated balance in an atopic mother influences the Th2-skewed balance of pregnancy, thereby influencing the maintenance of pregnancy. They evaluated this by analysing the number of children produced in relation to maternal atopy but did not analyse the number of preterm and term pregnancies. They found that atopic mothers had more children, from which they concluded that the maternal atopic genotype may be associated with an increased likelihood for successful pregnancy outcome. Sunyer et al. (2001) reached the opposite conclusion, reporting that maternal atopy was inversely related to the number of offspring produced.

In some studies evaluating perinatal factors and later atopy of the child, this subject has been highlighted in discussion. Bråbäck et al. (1998) suggested that in pregnancies terminating prematurely the foetoplacental Th2 cell skewing might be less pronounced. This may be related to the finding that the process of labour is mediated by proinflammatory cytokines (IL-8, TNF-α, IL-6 and IL-1β) (Peltier 2003).

While the association between maternal atopy and preterm birth has not been widely studied, the effect of maternal asthma on preterm delivery has. Maternal asthma

has been repeatedly linked to an increased risk of preterm delivery (Kelly et al. 1995, Kramer et al. 1995, Demissie et al. 1998, Liu et al. 2001, Källen et al. 2000). Kramer et al. (1995), however, found no association between metacholine responsiveness in the asthmatic mother and preterm delivery, concluding that non-atopic, non-cholinergic mechanisms may underlie bronchial and uterine smooth muscle lability. Some studies have found no relationship between maternal asthma and the course of pregnancy, especially when asthma is optimally controlled (Jana et al. 1995, Alexander et al. 1998, Minerbi-Codish et al. 1998). Asthma is, in any case, only partly attributable to atopy (Pearce et al. 1999). In the study of Burrows et al. (1989), 53% of adult asthma cases were attributable to atopy. Thus, possible associations between maternal atopy and maternal asthma and preterm delivery can be assumed to have different mechanisms.

In conclusion, atopic status of the mother during pregnancy may influence the maintenance of a successful pregnancy, providing some protection against preterm delivery. This association by leading to a lower genetic risk could also partly explain the decreased atopic sensitization rates in preterm children. The results of multivariate analyses, however, showed that prematurity also has an independent effect on atopic predisposition of preterm children.

METHODOLOGICAL CONSIDERATIONS

Study subjects

In studies I-III, the criterion for the preterm group was a birth weight of 1500 g or less. Thus, these subjects represented a group of VLBW children. A weight of 1500 g is normally reached in the Finnish population at a gestational age of 29-30 weeks (2 SD including weeks 28-32) (Pihkala et al. 1989). In the analyses, the mean gestational age of the group was found to be 28.5 (range 23.4-33.7) weeks. Thus, the group can also be deemed to represent a group of children born severely preterm. The designation “preterm” was preferred to the term “VLBW” in this study to emphasize the effect of immaturity rather than the effect of growth. However, with weight-based inclusion criteria and without adjusting weight to gestational age, children who have suffered from intrauterine growth retardation unavoidably become represented in the group. Nineteen children (26%) in the preterm group had a birth weight below -2 SD. The

possible effect of these children with intrauterine growth retardation on the group results cannot be estimated because of the relatively small group size, in addition to not knowing whether foetal disproportioned growth has an independent association with atopic sensitization.

The criterion for the full-term control group was a birth weight of over 2500 g. Gestational age data for the control group were unavailable. A weight of 2500 g is normally reached at the gestational age of 34 weeks (2 SD including weeks 32-38) (Pihkala et al. 1989). Based on gestational age, the preterm and full-term groups could have slightly overlapped. The mean birth weight of the full-term group here was, however, markedly higher, 3593 (SD 470) g, with only two children having a birth weight of less than 2900 g. Thus, the full-term group can be assessed as representing a group of children born at term, although the minimum cut-off for weight could have been higher.

Selection of the preterm group as 100 consecutively born children fulfilling the criteria was valid, but selection of the full-term group had some limitations. The children were invited from two Finnish-speaking schools in the Helsinki region, one from an urban and the other from a rural area. All children from four classes whose birth weight fulfilled the criterion were invited. Population-based random sampling from a birth cohort of the same time period and the same area as the preterm group would have been ideal. Congruence between our results of frequencies of atopy in the full-term group and results of population-based epidemiological studies on atopy in children shows, however, that the full-term group is representative of the general child population. Possible differences in background factors between the groups of preterm and full-term children were taken into account by performing multivariate logistic regression analysis related to the main outcome variables and by including possible confounding factors and risk factors for atopy in the model. The results of multivariate analysis did not change the outcome as compared with results of univariate analysis.

In the second part of the study, the selection of the groups of parents was valid, but the participation was low, 56%, and this may have limited the emergence of differences between the groups.

Study design and methods

Participation bias is always possible in a cohort study. However, the bias could be assumed to be similar in the preterm and full-term children, i.e. allergic individuals could be equally over-represented in both groups. The same argument can be applied to the participation of parents of preterm and full-term children.

The study was retrospective, which set limits on the assessment of data related to early feeding, onset of atopic and respiratory symptoms and infection morbidity. In a retrospective study, the possibility of recall bias also emerges. Individuals with a particular adverse health outcome may be more likely to identify, remember and report their exposure and illness than those non-exposed. The selection criteria of the groups and several main outcome measures of atopy (SPT, IgEs, ECP) were, however, precise and out of reach of recall bias, supporting the validity of the differences found between the groups.

Data collection may also be biased by an investigator who is aware of the study hypothesis and subjects' exposure status. This study was not blinded; the investigators and nurses knew the groupings and the study hypothesis. Possible bias related to this issue can, nevertheless, be considered minimal because the study was performed according to pre-defined diagnostic criteria and by standardized methods.

Certain limitations were, however, related to the performance of exercise spirometry. The exercise was performed in a non-standardized manner, by trampoline jumping. The objectives of the exercise were the same as in free outdoor running; a heart rate of >170/min for a 6-minute period, providing justification for the performance. Spirometry exhalation occuring just 10 minutes after the exercise was related to practical problems in carrying out the study.

Study design (I, II, III) included no prior sample size and power calculations. The sample size was relatively small increasing the probability of type II error (false negative finding). Analysis and interpretations of background factors within the groups particularly suffered from the small sample size. The sample size can, however, be judged to be sufficiently large, as significant differences were seen between the groups with regard to the main objectives of the study. In addition, odds ratios with confidence intervals reported for key outcomes allow the reader to quantify the power related to the results. These confidence intervals do justify the conclusions.

A limitation related to the study of parents of preterm and full-term children was the lack of data on background factors (e.g. smoking, socio-economic status, living area, pets); the questionnaire included only data on atopic symptoms and doctor-diagnosed atopic diseases in parents. Thus, the effect of possible confounding factors could not be taken into account in analysis by multivariate methods.

FUTURE ASPECTS

The results of differences in atopic findings and food antigen responses between children born preterm and those born full-term provide insight into the significance of early life in priming of immune responses, especially in development of tolerance. The role of immaturity in the process needs to be verified in future studies. These findings provide a framework for further research on early immunological mechanisms related to immaturity at birth.

The epidemiological finding of a possible association between maternal atopy and maintenance of pregnancy offers new data on the effect of chronic immune disease on course of pregnancy. Many epidemiological and immunological studies are, however, required to verify this relationship and to clarify the underlying immunopathogenesis.

CONCLUSIONS

This study was undertaken to evaluate the association between atopy and prematurity from two viewpoints. First, long-term effects of prematurity on the immune responses of the child were investigated in a group of 10-year-old children born severely preterm. A group of coeval children born at term served as controls. Second, the association between maternal atopy and premature birth was evaluated by comparing parents of VLBW children and parents of children with a birth weight above 3000 g.

The main conclusions of the study are as follows:

1. Significant prematurity at birth decreases the risk of later atopic sensitization. Immaturity of the immune system accompanied by a more permeable gut and qualitatively and quantitatively different early neonatal antigen exposure probably play a role in the maturation of immune responses.

2. Children born severely preterm are, by 10 years of age, at risk of recurrent wheezing and impaired lung function, especially those who have had respiratory problems and intensive care treatment during the neonatal period. Atopy in this group is not associated with the cumulative incidence of wheezing but is a prognostic factor for wheezing at school age.

3. Marked immaturity at birth has long-lasting consequences on food antigen-related immune responses. Children born preterm produce at the age of 10 years markedly lower levels of antibodies to early-introduced food antigens, possibly reflecting increased susceptibility to develop tolerance.

4. Maternal atopic immune balance may interact with and strengthen foetoplacental Th2 cell responses, thus supporting the maintenance of pregnancy.

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